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Anti-HER2/neu IgG3 heavy chain-IFN $\alpha$ 

M G W S W V M H L S P V S N C G V H S Q V Q L V Q S G A E V K K P G E  
S L K I S C K G S G Y S F T S Y W I A W V R Q M P G K G L E Y M G L I Y  
P G D S D T K Y S P S F Q G Q V T I S V D K S V S T A Y L Q W S S L K P  
S D S A V Y F C A R H D V G Y C T D R T C A K W P E Y F Q H W G Q G T  
L V T V S S A S T K G P S V F P L A P C S R S T S G G T A A L G C L V K  
D Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S S G L Y S L S  
S V V T V P S S S L G T Q T Y T C N V N H K P S N T K V D K R V E L K T  
P L G D T T H T C P R C P E P K S C D T P P P C P R C P E P K S C D T P  
P P C P R C P E P K S C D T P P P C P R C P A P E L L G G P S V F L F P  
P K P K D T L M I S R T P E V T C V V V D V S H E D P E V Q F K W Y V D  
G V E V H N A K T K L R E E Q Y N S T F R V V S V L T V L H Q D W L N G  
K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E P Q V Y T L P  
P S R E E M T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E  
N N Y N T T P P M L D S D G S F F L Y S K L T V D K S R W Q Q G N I F S  
C S V M H E A L H N H Y T Q K S L S L S P G K S G G G Q S G G G G S G  
G G Q S C D L P Q T H N L R N K R A L T L L V Q M R R L S P L S C L K D  
R K D F G F P Q E K V D A Q Q I K K A Q A I P V L S E L T Q Q I L N I F T  
S K D S S A A W N A T L L D S F C N D L H Q Q L N D L Q G C L M Q Q V  
G V Q E F P L T Q E D A L L A V R K Y F N R I T V Y L R E K K H S P C A  
W E V V R A E V W R A L S S S A N V L G R L R E E K

Anti-HER2/neu IgG3-IFN $\alpha$  light chain sequence

M E W S C V M L F L L S V T A G V H S D I Q M T Q S P S S L S A S V G D  
R V T I T C R A S Q D V N T A V A W Y Q Q K P G K A P K L L I Y S A S F  
L Y S G V P S R F S G S R S G T D F T L T I S S L Q P E D F A T Y Y C Q  
Q H Y T T P P T F G Q Q G T K V E I K R T V A A P S V F I F P P S D E Q L K  
S G T A S V V C L L N N F Y P R E A K V Q W K V D N A L Q S G N S Q E  
S V T E Q D S K D S T Y S L S S T L T L S K A D Y E K H K V Y A C E V T  
H Q G L L S S P V T K S F N R G E C

*Fig. 1A*

**αCD20 light chain - nucleic acid sequence**

ATGAAGTTGCCCTGTTAGGCTGTTGGTGTGATGTTCTGGATTCCCTGCCTCCAGCAGTC  
TTGTTCTCTCCCAGTCTCCAGCAATCCTGTCGCACTCCAGGGGAGAAGGTACAATGAC  
TTGCAGGGCCAGCTCAAGTGTAAAGTTACATCCACTGGTCCAGCAGAAGCCAGGATCCTCC  
CCCAAACCCCTGGATTATGCCACATCCAACCTGGCTCTGGAGTCCTGTTCGCTTCAGTG  
GCAGTGGGTCTGGGACTTCTTAECTCTCACAATCAGCAGACTGGAGGGCTGAAGATGCTGC  
CACTTATTACTGCCAGCAGTGGACTAGTAACCCACCCACGTTCCGGAGGGGGGACCAAGCTG  
GAAATCAAACGTAAGTCGACTTTCTCATCTTTTTATGTGAAGACACAGGTTTTCATGT  
TAGGAGTTAAAGTCAGTTCAGAAAATCTTGAGAAAATGGAGAGGGCTCATTATCAGTTGAC  
GTGGCATAACAGTGTCAAGATTTCTGTTATCAAGCTAGTGAGATTAGGGCAAAAGAGGC  
TTTAGTTGA

**αCD20 light chain - amino acid sequence**

MKLPVRLLVLMFWIPASSSQIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSS  
PKPWIYATSNLASGPVVRSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKL  
EIKRKSTFSSFFMCKTQVFMLGVKVSENLEKMERAHYQLTWHTVSDFLIKLVLRLGAKRG  
FS

***Fig. 1B***

**αCD20-IgG3-muIFNα Gly<sub>4</sub>Ser linker - nucleic acid sequence**

ATGTACTTGGGACTGAACGTGTAATCATAGTTTCTCTAAAAGGTGTCCAGAGTCAGG  
TACAAC TGCAGCAGCCTGGGCTGAGCTGGTGAAGCCTGGGCCTCAGTGAAGATGTCCTG  
CAAGGCTTCTGGCTACACATTACCA GTTACAATATGCACTGGTAAAACAGACACCTGGT  
CGGGGCCTGGAATGGATTGGAGCTATTATCCC GGAAATGGTGTAACTTCCCTACAATCAGA  
AGTTCAAAGGCAAGGCCACATTGACTGCAGACAAAT CCTCCAGCACAGCCTACATGCAGCT  
CAGCAGCCTGACATCTGAGGA C TCTGCGGTCTATTACTGTGCAAGATGCACTTACACGGC  
GGTGA C TGGTACTTCAATGTCTGGGGCGCAGGGACCACGGTCACCGTCTCTGCAGCTAGCA  
CCAAGGGCCC ATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCTGGGGCACAGC  
GGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTGTTGAACTCA  
GGC GCCCTGACCAGCGCGTGCACACCTTCCGGCTGTCTACAGTCTCAGGACTCTACT  
CCCTCAGCAGCGTGGTGACCGTGCCTCCAGCAGCTGGCACCCAGACCTACAC TGCAA  
CGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGAGTTGGTGAGAGGCCAGCGCAG  
GGAGGGAGGGTGTCTGCTGGAAGCCAGGCTCAGCCCTCTGCTGGACGCATCCGGCTGT  
GCAGTCCCAGCCCAGGGCACCAAGGCAGGCCCTGTGACTCTCACCAGGCTCCGGCAGGC  
CCGCCCCACTCATGCTCAGGGAGAGGGTCTTCTGGCTTTCCACCAGGCTCCGGCAGGC  
ACAGGCTGGATGCCCTACCC CAGGCCCTCACACACAGGGCAGGTGCTGCGCTCAGAGC  
TGCCAAGAGCCATATCCAGGAGGACCTGCCCTGACCGAGCTCAA A A C C C A C T T G G T G A  
CACAACTCACACATGCCACGGTCCCAGAGCCAAATCTTGTGACACACCTCCCCGTGC  
CCAAGGTGCCAGAGCCAAATCTTGTGACACACCTCCCCGTGCCAAGGTGCCAGAGC  
CCAAATCTTGTGACACACCTCCCCGTGCCAAGGTGCCATGATTCCGGACCCCTGAG  
GTCACGTGCGTGGTGGGACGTGAGCCACGAAGACCCGAGGTCCAGTTCAAGTGGTACG  
TGGACGGCGTGGAGGTGATAATGCCAAGACAAAGCTGCGGGAGGAGCAGTACAACAGCAC  
GTTCCGTGTGGTCAGCGTCCCTCACCGTCTGCACCA GGTACTGGCTGAACGGCAAGGAGTAC  
AA GTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCATCGAGAAACCATCTCAAAGCCAA  
AATGACCAAGAACCAAGGTGAGCCTGACCTGCCCTGGTCAAAGGCTCTACCCAGCGACATC  
GCCGTGGAGTGGGAGAGCAATGGCAGCCGGAGAACAACTACAACACCAGCCTCCATGC  
TGGACTCCGACGGCTCCTCTTCCCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCA  
GCAGGGGAACATCTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACC ACTACACGCAG  
AAGAGCCTCTCCCTGTCTCCGGTAAATCTGGTGGCGGTGGATCCTGTGACCTGCTCAGA  
CTCATAACCTCAGGAACAAGAGAGGCCTTGACACTCCTGGTACAAATGAGGAGACTCTCCCC  
TCTCTCCTGCCCTGAAGGACAGGAAGGACTTTGGATTCCCGCAGGAGAAGGTGGATGCCAG  
CAGATCAAGAAGGCTCAAGCCATCCCTGTCTGAGT GAGCTGACCCAGCAGATCTGAACA  
TCTTCACATCAAAGGACTCATCTGCTGCTTGGAATGCAACCCCTCTAGACTCATTCTGCAA  
TGACCTCCACCAGCAGCTCAATGACCTGCAAGGTTGTCTGATGCAGCAGGTGGGGTGCAG  
GAATT TCCCTGACCCAGGAAGATGCCCTGCTGGCTGTGAGGAAATACTTCCACAGGATCA  
CTGTGTACCTGAGAGAGAAAGAACACAGCCCTGTGCCCTGGGAGGTGGTCA GAGCAGAAGT  
CTGGAGAGCCCTGTCTTCCCTGCCAATGTGCTGGGAAAGACTGAGAGAAGAGAAATGA

***Fig. 1C***

αCD20-IgG3-muIFNα Gly<sub>4</sub>Ser linker - Amino acid sequence

MYLGLNCVIIIVFLLKGVQSQVQLQQPGAEVLKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNWGAGTTVTVAIASTKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVNHKPNTKVDKRVGERPAQGGRVSAGSQAQPSCLDASRLCSPSPGHQGRPRLTPHEASARPHTAQGEGLLAFSTRLAGTGWMPLPQALHTQGQVLRSELPRAISSRTIPLTELKTPLGDTTHTCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPMISRTPEVTCVVVDVSHEDPEVQFKWYVDGVEVHNAKTKLREEQYNSTFRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISAKMTKNQVSLTCLVKGFYPSDIAVEWESNGQOPENNYNTTPMLSDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNHYTQKSLSLSPGKSGGGGSCDLPQTHNLRNKRALTLLVQMRRRLSPLSCLKDRKDGFQEKVDAQQIKKAQAIIPVLSLTQQILNIFTSKDSSAAWNATLLDSFCNDLHQQLNDLQGCLMQQVGVQEFLTQEDALLAVRKYFHRITVYLREKKHSPCAWEVVRAEVWRALSSSANVLGRLREEK

*Fig. 1C, cont'd.*

**αCD20-IgG3-muIFNα alpha helical linker - nucleic acid sequence**

ATGTACTTGGGACTGAACCTGTATACTATAGTTTCTCTAAAAGGTGTCCAGAGTCAGG  
TACAACCTGCAGCAGCCTGGGCTGAGCTGGTGAAGCCTGGGCCTCAGTGAAGATGTCTG  
CAAGGCTTCTGGCTACACATTACAGTTACAATATGCACTGGTAAACAGACACCTGGT  
CGGGGCCTGGAATGGATTGGAGCTATTATCCCGAAATGGTGTAACTTCCTACAATCAGA  
AGTTCAAAGGCAGGCCACATTGACTGCAGACAAATCCTCCAGCACGCCATGAGCT  
CAGCAGCCTGACATCTGAGGACTIONTCGGTCTATTACTGTCAAGATCGACTTACCGC  
GGTGACTIONGGTACTTCAATGTCTGGGCGCAGGGACCACGGTCACCGTCTGCAGCTAGCA  
CCAAGGGCCCATCGGTCTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCTGGGGCACAGC  
GGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCAACCGGTGACGGTGTGTTGAACTCA  
GGCGCCCTGACCAGCGCGTGCACACCTCCCGTGTCTACAGTCCTCAGGACTCTACT  
CCCTCAGCAGCGTGGTGACCGTGCCTCCAGCAGCTTGGCACCCAGACCTACACCTGCAA  
CGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGGTGTGAGAGGCCAGCGCAG  
GGAGGGAGGGTGTCTGCTGGAAGGCAGGCTCAGCCCTCTGCCTGGACGCATCCGGCTGT  
GCAGTCCCAGCCCAGGGCACCAAGGCAGGCCCTGACTCTCACCCGGAGGCCTCTGC  
CCGCCCACTCATGCTCAGGGAGAGGGTCTCTGGCTTTTACCCAGGCTCCGGCAGGC  
ACAGGCTGGATGCCCTACCCAGGCCCTCACACACAGGGCAGGTGCTGCGCTCAGAGC  
TGCCAAGAGCCATATCCAGGAGGACCTGCCCTGACCGAGCTAAAACCCACTTGGTGA  
CACAACCTCACACATGCCACGGTCCCAGAGCCAAATCTTGTGACACACCTCCCCGTGC  
CCAAGGTGCCAGAGCCAAATCTTGTGACACACCTCCCCGTGCCAAGGTGCCAGAGC  
CCAAATCTTGTGACACACCTCCCCGTGCCAAGGTGCCATGATTCCGGACCCCTGAG  
GTCACGTGCGTGGTGGACGTGAGCCACGAAGACCCGAGGTCCAGTTCAAGTGGTACG  
TGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCTGCGGGAGGAGCAGTACAACAGCAC  
GTTCCGTGTGGTCAGCGTCTCACCGTCTGCACCAGGACTGGCTGAACGGCAAGGAGTAC  
AAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCATCGAGAAAACCATCTCAAAGCCA  
AAATGACCAAGAACCAAGGTGACCTGCCTGGTCAAAGGCTCTACCCAGCGACAT  
CGCCGTGGAGTGGAGAGCAATGGCAGCCGGAGAACAAACTACAAACACCACGCCCTCCATG  
CTGGACTCCGACGGCTCTTCTCCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGC  
AGCAGGGGAACATCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACACGCA  
GAAGAGCCTCTCCCTGCTCCGGTAAAGCAGAGGCCAGCTAAAGAGGCCAGCAGCCAAA  
GCCGGATCCTGTGACCTGCCTCAGACTCATAACCTCAGGAACAAGAGAGGCCTTGACACTCC  
TGGTACAAATGAGGAGACTCTCCCTCTCCTGCCTGAAGGACAGGAAGGACTTGGATT  
CCCGCAGGAGAAGGTGGATGCCAGCAGATCAAGAAGGCTCAAGCCATCCCTGTGCTGAGT  
GAGCTGACCCAGCAGATCCTGAACATCTTACATCAAAGGACTCATCTGCTGCTGGATG  
CAACCCCTCTAGACTCATTCTGCAATGACCTCCACCAGCAGCTCAATGACCTGCAAGGTTG  
TCTGATGCAGCAGGTGGGGTGCAGGAATTCCCTGACCCAGGAAGATGCCCTGCTGGCT  
GTGAGGAAATACTCCACAGGATCACTGTGTACCTGAGAGAGAAGAAACACAGCCCTGTG  
CCTGGGAGGTGGTCAGAGCAGAAGTCTGGAGAGGCCCTGCTTCCCTGCCAATGTGCTGGG  
AAGACTGAGAGAAGAGAAATGA

***Fig. 1D***

**αCD20-IgG3-muIFNα alpha helical linker - amino acid sequence**

MYLGLNCVIIVFLLKGVQSQVQLQQPGAEVLVKPGASVKMSCKASGYTFTSYNMHWVKQTPG  
RGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSTAYMQLSSLTSEDSAVYYCARSTYYG  
GDWYFNWGAGTTVTVAIASTKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPVTWSWNS  
GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVNHKPNTKVDKRVGERPAQ  
GGRVSAGSQAQPSCLDASRLCSPSPGHQGRPRLTPHEASARPHTAQGEGLLAFSTRLAG  
TGWMPLPQALHTQGQVLRSELPRAlSRRTLPLTELKTPLGDTHTCPRCPEPKSCDTPPPC  
PRCPEPKSCDTTPPCPRCPEPKSCDTTPPCPRCPMISRTPEVTCVVVDVSHEDPEVQFKWY  
VDGVEVHNAKTKLREEQYNSTFRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA  
KMTKNQVSLTCLVKGFYPSDIAVEWESENQOPENNYNTTPMLSDGSFLYSKLTVDKSRW  
QQGNIFSCSVMHEALHNHTQKSLSLSPGK**AEEAAKEAAAKA**GSCDLPQTHNLRNKRALTL  
LVQMRRRLSPLSCLKDRKDFGFPQEKVDAQQIKKAQAIPLSELTQQILNIFTSKDSSAAWN  
ATLDSFCNDLHQQLNDLQGCLMQQGVQEFPLTQEDALLAVRKYFHRTVYLREKKHSPC  
AWEVVRAEVWRALSSSANVLGRLREKK

***Fig. 1D, cont'd.***

**αCD20-IgG3-huIFNα Gly<sub>4</sub>Ser linker - nucleic acid sequence**

ATGTACTTGGGACTGAACCTGTGTAATCATAGTTTCTCTAAAAGGTGTCCAGAGTCAGG  
TACAACITGCAGCAGCCTGGGCTGAGCTGGTGAAGCCTGGGGCTCAGTGAACATGTCTG  
CAAGGCTTCTGGCTACACATTACCAAGTACAATAATGCCACTGGTAAACAGACACCTGGT  
CGGGGCCTGGAATGGATTGGAGCTATTATCCCCGAAATGGTGATACTTCTACAATCAGA  
AGTCAAAGGCAAGGCCACATTGACTGCAGACAAATCCTCCAGCACAGCCTACATGCAGCT  
CAGCAGCCTGACATCTGAGGACTCTGCGGTCTATTACTGTGCAAGATCGACTTACTACGGC  
GGTGAUTGGTACTTCAATGTCTGGGCGCAGGGACCACGGTCACCGTCTCTGCAGCTAGCA  
CCAAGGGGCCATCGGTCTTCCCCCTGGGCCCTGCTCCAGGAGCACCTCTGGGGCACAGC  
GGCCCTGGGCTGGCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTGTTGAACTCA  
GGGCCCTGACCAGCGCGTGCACACCTCCCCGTGTCCTACAGTCTCAGGACTCTACT  
CCCTCAGCAGCGTGGTACCGTCCCCCTCCAGCAGCTTGGCACCCAGACCTACACCTGCAA  
CGTGAATCACAAGGCCAGCAACACCAAGGTGGACAAGAGAGTTGGTGAGAGGCCAGCGCAG  
GGAGGGAGGGTGTGCTGGAAGCCAGGCTCAGCCCTCTGCTGACTCTCACCCGGAGGCTCTG  
GCAGTCCCAGCCCAGGGCACCAAGGCAGGCCCTGCTGACTCTCACCCGGAGGCTCTG  
CCGCCCCACTCATGCTAGGGAGAGGGTCTCTGGTTTCTCACCAGGCTCCGGCACGG  
ACAGGCTGGATGCCCTACCCAGGCCCTCACACACAGGGCAGGTGCTGCGCTCAGAGC  
TGCCAAGAGCCATATCCAGGAGGACCTGGCCCTGACCGAGCTAAAAACCCACTTGGTGA  
CACAACTCACACATGCCACGGTGCCTCAGAGCCAAATCTTGTGACACACCTCCCCGTG  
CCAAGGTGCCAGAGCCAAATCTTGTGACACACCTCCCCGTGCCAACGGTGCCAGAGC  
CCAAATCTTGTGACACACCTCCCCGTGCCAACGGTCCCCATGATTCCCCGGACCCCTGAG  
GTCACGTGCGTGGTGGGACGTGAGCCACGAAGACCCGAGGTCCAGTTCAAGTGGTAOG  
TGGACGGCGTGGAGGTGCTAAATGCCAACAGACAAAGCTGCCAGGAGCAGTACAACAGCAC  
GTTCGGTGTGGTCAACCTCTCACCGTCTGACCCAGACTGCCAACCCCCAACGGACTAC  
AAAGTCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATGAGAAACCATCTCAAAGGCCAA  
AATGACCAAGAACCAAGGTCAAGCTGACCTGCCCTGGTCAAAGGCTCTACCCAGCGACATC  
GCCGTGGAGTGGAGAGCAATGGCAGGCCGGAGAACAACTAACACACCACCCCTCCCATG  
TGGACTCCGACGGCTCCCTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCA  
GCAGGGAACATCTTCTCATGCTCCGTATGCAATGAGGCTCTGCACAACCAACTACACGCA  
AAGAGCCTCTCCGTCTCCGGTAAATCTGGTGGCGGTGGATCTGTGATCTGCCTCAA  
CCCACAGCTGGTAGCAGGAGGACCTTGATGCTCTGGTACAGATGAGGAGAACATCTCT  
TTTCTCTGCTGAAGGACAGACATGACTTGGATTCCCCAGGAGGAGTTGGCAACCAG  
TTCCAAAAGGCTGAAACCATCCCTGTCTCCCATGAGATGATCCAGCAGATCTCAATCTCT  
TCAGCACAAAGGACTCATCTGCTGCTTGGATGAGACCCCTCTAGACAAATTCTACACTGA  
ACTCTACCAAGCAGCTGAATGACCTGGAAAGCCTGTGATACAGGGGGTGGGGGTGACAGAG  
ACTCCCCCTGATGAAGGAGGACTCCATTCTGGCTGTGAGGAAATACTTCCAAAGAACATC  
TCTATCTGAAAGAGAACATACAGCCCTGTGCCTGGAGGTTGTCAAGAGCAGAAATCAT  
GAGATCTTTCTTGTCAACAAACTGCAAGAAAGTTAAGAAGTAAGGAATGA

***Fig. 1E***

**αCD20-IgG3-huIFNα Gly<sub>4</sub>Ser linker - amino acid sequence**

MYLGLNCVIIIVFLLKGVQSQVQLQQPGAEILVKPGASVKMSCKASGYTFTSYNMHWVKQTPG  
RGLEWIGAIYPGNQDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYG  
GDWYFNVWGAGITTVSAASTKGPVFPLAPCSRSTS GGTAALGCLVKDYFPEPVTVSWNS  
GALTSGVHTEPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVNHKPSNTKVDKRVGERPAQ  
GGRVSAGSQAQPSCLDASRLCSPGPQHQGRPRLTPHEASARPHTAQGEGLLAFSTFLRAG  
TGWMPLPQALHTQGQVLRSELPRAlSRRTIPLTELKTPLGDTHTCPRCPEPKSCDTPPP  
PRCPEPKSCDTPPPCCPRCPEPKSCDTPPPCCPRCPMISRTPEVTCVVVDVSHEDPEVQFKWY  
VDGVEVHNNAKTKLREEQYNSTFRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA  
KMTKNQVSLTCLVKGFYPSDIAVEWESENQOPENNYNTTPMLSDGSFFLYSKLTVDKSRW  
QQGNIFSCSVMHEALHNHYTQKSLSLSPGKSGGGGSCDLPQTHSLGSRRTLMLLAQMRRIS  
LFSCCLKDRHDFGFQEEFGNQFQKAETIPVLHEMIQQIFNLFSTKUSSAAWDETILLDKFYT  
ELYQQLNDLEACVIQGVGVIEPILMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEI  
MRSFSLSTNLQESLRSKE

***Fig. 1E, cont'd.***

**αCD20-IgG3-huIFNα alpha helical linker - nucleic acid sequence**

ATGTACTTGGGACTGAACITGTGAATCATAGTTTCTCTTAAAGGTGTCCAGAGTCAGG  
TACAACITGCAGCAGCCTGGGCTGAGCTGGTGAAGCCTGGGCCTCAGTGAAGAIGTCCTG  
CAAGGCTTCTGGCTACACATTACCAATATGCACTGGTAAACACAGACACCTGGT  
CGGGGCCTGGAATGGATTGGAGCTATTATCCCAGAAATGGTGATACTTCCCTACAATCAGA  
AGTTCAAAGGCAAGGCCACAITGACTGCAGACAAATCCTCCAGCACAGCCTACATGCAGCT  
CAGCAGCCTGACATCTGAGGACTCTGCGGTCTATTACTGTGCAAGATCGACTTACTACGGC  
GGTGAUTGGTACTTCAATGTCTGGGCGCAGGGACCACGGTCACCGTCTCTCCAGCTAGCA  
CCAAGGGGCCATCGGTCTCCCCCTGGCCCCCTGCTCCAGGAGCACCTCTGGGGCACAGC  
GGCCCTGGGCTGCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTGGAACCTCA  
GGCGCCCTGACCAGCGCGTGCACACCTTCCCGCTGCTCCAGCTGGCACCCAGACCTACACCTGCAA  
CGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTGGTGAGAGGCCAGCGCAG  
GGAGGGAGGGTGTCTGCTGGAAAGCCAGGCTCAGCCCTCTGGCTGGACGCATCCCCGCTGT  
GCAGTCCCAGGCCAGGGCACCAAGGCAGGGCCGGTGTGACTCTCACCGGGAGGCCTCTGC  
CCGCCCTACTCATGCTCAGGGAGAGGGTCTCTGGCTTTTCCACCAAGGCTCGGGCACAGC  
ACAGGCTGGATGCCCTACCCAGGCCCTCACACACAGGGCAGGTGCTGCGCTCAGAGC  
TGCGAACAGAGOCATATCCAGGAGGACCTGGCCCTGACCGAGCTAAAACCCCAACTGGTGA  
CACAACTCACACATGCCACGGTGCACAGAGCCAAATCTTGTGACACACACCTCCCCGTGC  
CCAAGGTGCCAGAGCCAAATCTTGTGACACACCTCCCCGTGCCAAGGTGCCAGAGC  
CCAATCTTGTGACACACCTCCCCGTGCCAAGGTGCCATGATTCCCCGACCCCTGAG  
GTCACGTGCGTGGTGGACGTGAGCCACGAAGACCCGAGGTCCAGTTCAAGTGGTACG  
TGGACGGCGTGGAGGTGCTAAATGCGAACAAAGCTGCGGGAGGAGCAGTACAACACGAC  
GTTCCGTGCGTCAACCGTCTCACCCTGACCCAGACTGGCTGAACGGCAAGGACTAC  
AAGTGCAAGGTCTCCAACAAAGCCCTCCAGCCOCATCGAGAAAACCATCTCCAAGCCA  
AAATGACCAAGAACAGGTCAGCCAGACCTGGCTGGTCAAAGGCTCTACCCAGCGACAT  
CGCCGTGGAGTGGAGAGCAATGGGAGCGCGAGAACACTACAAACACCCACGCCCTCCATG  
CTGGACTCCGACGGCTCTTCTTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGC  
AGCAGGGGAACATCTTCTCATGCTCCGTGATGCGATGAGGCTCTGCAACAACCAACTACCGCA  
GAAGAGCCTCTCCCTGCTCCGGTAAATCTGGTGGCGGTGGATCTGTGATCTGGCTCAA  
ACCCACAGCCTGGGTAGCAGGAGGACCTGATGCTCTGGCACAGATGAGGAGAATCTCTC  
TTTCTCTGCTGAAGGACAGACATGACTTGGATTCCCCAGGAGGAGTTGGCAACCA  
GTTCCAAAAGGCTGAAACCAACCTGCTCCATGAGATGATCCAGGAGATCTTCAATCTC  
TTCAGCACAAAGGACTCATCTGCTGCTTGGGATGAGACCCCTCTAGACAAATTCTACACTG  
AACTCTACCAAGCAGCTGAATGACCTGGAAGCCTGTGATAACAGGGGTGGGGTGCAGAGA  
GACTCCCTGATGAAGGAGGACTCCATTCTGGCTGTGAGGAATAACTTCCAAAGAATCA  
CTCTATCTGAAAGAGAAGAAATACAGCCCTGTGCCTGGAGGTTGTCAGAGCAGAAATCA  
TGAGATCTTTCTTGTCAACAAACTTGCAAGAAAGTTAAGAAGTAAGGAATGA

***Fig. 1F***

**αCD20-IgG3-huIFNα alpha helical linker - amino acid sequence**

MYLGLNCVIIIVFLLKGVQSQVQLQQPGAEVLVKGASVKMSCKASGYTFTSYNMHWVKQTPG  
RGLEWIGATYPNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVIDYCARSTYYG  
GDWYFNVWGAGTTVTVAASSTKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPVTVSWNS  
GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVNHKP SNTKVDKRVGERPAQ  
GGRV3AGSQAQPSCLDASRLCSPSPGHQGRPRLTPHPEASARP THAQGEGLLAFSTRLRAG  
TGWMPLPQALHTQGQVLRSELPRAISSRTLPLTELKTPLGDTHTCPRCPEPKSCDTEPPC  
PPCPEPKSCDTEPPCPRCPEPKSCDTPPPCPRCPMISRTPEVTCVVVDVSHEDPEVQFKWY  
VDGVEVHNNAKTKLREEQYNSTFRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA  
KMTKNQVSLTCLVKGFYPSDIAVEWESNGQOPENNYNTTPMLDSDGSFFLYSKLTVDKSRW  
QQGNITFSCSVVMHEALHNHYTOKSLSLSPGKSAEAAKEAAKACDLPOQTHSLGSRTIML  
AQMRRIISLFSCLKDRHDFGFPQEEFGNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDET  
LLDKFYTELYQQLNDEACVIQGVGVTEFLMKEDSILAVRKYFQRITLYLKERKYSPCAW  
EVVRAEIMRSFSLSTNLQESLRSKE

*Fig. 1F, cont'd.*

**αCD20-IgG1-muIFNα Gly<sub>4</sub>Ser linker - nucleic acid sequence**

ATGTACTTGGGACTGAACCTGTATACTAGTTTCTCTAAAAGGTGTCCAGAGTCAGGTACAAC  
TGCAGCAGCCTGGGCTGAGCTGGTGAAGCCTGGGCCTCAGTGAAGATGTCTGCAAGGCTCTGG  
CTACACATTACCAGTTACAATATGCACTGGTAAAACAGACACCTGGTCGGGCCTGGAATGGATT  
GGAGCTATTATCCCGAAATGGTATACTTCTACAATCAGAAGTTCAAAGCAAGGCCACATTGA  
CTGCAGACAAATCCTCCAGCACAGCCTACATGCAGCTCAGCAGCCTGACATCTGAGGACTCTGCGGT  
CTATTACTGTGCAAGATGACTTACAGCGGTGACTGGTACTTCAATGTCTGGGCGCAGGGACC  
ACGGTCACCGTCTGCAGCTAGCCAACCAAGGGCCCATCGGTCTCCCCCTGGCACCCCTCCCAA  
GAGCACCTCTGGGGCACAGCGGCCCTGGCTGCCTGGTCAAGGACTACTTCCCAGACCGGGAGCC  
CAAATCTTGTGACAAACTCACACATGCCAACCGTGCCCATGATCTCCGGACCCCTGAGGTACA  
TGCCTGGTGGACGTGAGCCACGAAGACCCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGG  
AGGTGCATAATGCCAAGACAAGCCGGAGGAGCAGTACAACAGCACGTACCGGGTGGTACGCCT  
CCTCACCGTCCTGCACCAGGACTGGTGAATGCAAGGAGTACAAGTGCAAGGTCTCAAACAAAGCC  
CTCCCAGCCCCATCGAGAAAACATCTCAAAGCAAAGGTGGACCCGTGGGTGCGAGGGCCAC  
ATGGACAGAGGCCGGCTGGCCACCCCTGCGCTGGAGAGTGACCGCTGTACCAAACCTCTGCTTAC  
AGGGCAGCCCCGAGAACACAGGTGTACACCCCTGCCCATCCGGGATGAGTGACCAAGAACAG  
GTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCAGCATGCCGTGGAGTGACCGCTGT  
GGCAGCCGGAGAACAACTACAAGACCACGCCTCCGTGCTGGACTCCGACGGCTCTTCTCCTCTA  
CAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGAACGTCTCTCATGCTCCGTGATGCAT  
GAGGCTCTGCACAACCACACGCAGAACAGGCCCTCTCCGTCTCCGGTAAATCTGGTGGCGGTG  
GATCCTGTGACCTGCCTCAGACTCATAACCTCAGGAACAAGAGAGCCCTGACACTCCTGGTACA  
GAGGAGACTCTCCCTCTCCTGCCTGAAGGACAGGAAGGACTTGGATTCCGCAGGAGAACGGT  
GATGCCAGCAGATCAAGAAGGCTCAAGCCATCCCTGTCTGAGTGAGCTGACCCAGCAGATCCTGA  
ACATCTCACATCAAAGGACTCATCTGCTGCTGGAATGCAACCCCTCTAGACTCATTGCAATGA  
CCTCCACCAGCAGCTCAATGACCTGCAAGGTTGCTGATGCAGCAGGTGGGGTGCAGGAATTCCC  
CTGACCCAGGAAGATGCCCTGCTGGTGTGAGGAATACTCCACAGGACTGTGACCTGAGAG  
AGAAGAACACAGCCCTGTGCCTGGAGGTGGTCAAGAGCAGAACGTCAGAGAGCCCTGCTTCTC  
TGCCAATGTGCTGGGAAGACTGAGAGAACGAAATGA

**αCD20-IgG1-muIFNα Gly<sub>4</sub>Ser linker - amino acid sequence**

MYLGLNCVIIVFLLKGVQSVQLQQPGAEVLVKPGASVKMSCKASGYTFTSYNMHWVKQTPG  
RGLEWIGAIYPNGDTSYNQKFKGKATLTADKSSTAYMQLSSLTSEDAVYYCARSTYYG  
GDWYFNVWGAGTTVTVAASQPRAHRSSPWHPPRPLGAQRPWAAWSRTTSPNREP KSCD  
KTHTCPPCPMISRTPETCVVVDVSHEDEPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV  
SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIISKAKGGTRGVRGPHGQRPARPTLCPESD  
RCTNLCP TGQPQREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT  
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGKS**GGGGS**C  
DLPQTHNLRNKRALTLLVQMRRRLSPLSCLKDRKDFGFPQEKVDAQQIKKAQAIPLVSELTQ  
QILNIFTSKDSAAWNATLLDSFCNDLHQQLNDLQGCLMQQVGVQEFPLTQEDALLAVRKY  
FHRI TVYLREKKHSPCAWEVVRAEVWRALSSSANVIGRLREEK

***Fig. 1G***

**αCD20-IgG1-muIFNα alpha helical linker - nucleic acid sequence**

ATGTACTTGGGACTGAACGTGTAATCATAGTTCTCTAAAAGGTGTCCAGAGTCAGGTACAAC  
TGCAGCAGCCTGGGGCTGAGCTGGTGAAGCCTGGGCCTCAGTGAAGATGTCTGCAAGGCTCTGG  
CTACACATTACCAGTTACAATATGCACTGGTAAAACAGACACCTGGTGGGCCTGGAATGGATT  
GGAGCTATTATCCCAGAATGGTGTAACTTCCTACAATCAGAAGTTCAAAGGCAAGGCCACATTGA  
CTGCAGACAAATCCTCCAGCACGCCTACATGCAGCTCAGCAGCCTGACATCTGAGGACTCTCGGGT  
CTATTACTGTGCAAGATCGACTTACTACGGCGGTGACTGGTACTTCAATGTCTGGGCGCAGGGACC  
ACGGTCACCGTCTGCAGCTAGCCAACCAAGGGCCATCGGTCTTCCCCCTGGCACCCCTCCCAA  
GAGCACCTCTGGGGCACAGCGGCCCTGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGGAGCC  
CAAATCTGTGACAAAACATCACACATGCCAACCGTGCCAATGATCTCCGGACCCCTGAGGTAC  
TGCCTGGTGGTGGACGTGAGCCACGAAGACCCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGG  
AGGTGCATAATGCCAAGACAAAGCCGGAGGAGCAGTACAACAGCACGTACCGGTGGTCAAGGCT  
CCTCACCGTCTGCACCAGGACTGGCTGAATGCCAAGGAGTACAAGTGCAAGGTCTCAAACAAAGCC  
CTCCCAGCCCCATCGAGAAAACCATCTCCAAGGCCAAGGTGGGACCCGTGGGTGCGAGGGCCAC  
ATGGACAGAGGCCGGCTGGCCCACCCCTGCCCTGAGAGTGACCGCTGTACCAACCTCTGCCTAC  
AGGGCAGCCCCGAGAACACAGGTGTACCCCTGCCCTGCCCTGAGAGTGACCAAGAACAG  
GTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATGCCGTGGAGTGAGGAGCAATG  
GGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGTGGACTCCGACGGCTCTTCTCA  
CAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCAT  
GAGGCTCTGCACAACCAACTACACGCAGAACAGGCTCTCCCTGTCTCCGGTAAATCTGGTGGCGGTG  
GATCCTGTGACCTGCCTCAGACTCATACCTCAGGAACAAGAGAGCCTGACACTCCTGGTACAAAT  
GAGGAGACTCTCCCTCTCCTGCCCTGAAGGACAGGAAGGACTTGGATTCCCGCAGGAGAACGGT  
GATGCCAGCAGATCAAGAAGGCTCAAGCCATCCCTGTCTGGTGAAGTGACCCAGCAGATCCTGA  
ACATCTTCACATCAAAGGACTCATCTGCTGTTGGAATGCAACCCCTCTAGACTCATTGCAATGA  
CCTCCACCAGCAGCTCAATGACCTGCAAGGTTGTGATGCAGCAGGTGGGGTGCAGGAATTCCC  
CTGACCCAGGAAGATGCCCTGCTGGCTGTGAGGAAATACTCCACAGGATCACTGTGTACCTGAGAG  
AGAAGAAACACAGCCCTGTGCCTGGGAGGTGGTCAAGCAGCAGAACGTCTGGAGAGGCCCTGTCTCCTC  
TGCCAATGTGCTGGGAAGACTGAGAGAACGAAATGA

**αCD20-IgG1-muIFNα alpha helical linker - amino acid sequence**

MYLGLNCVIIIVFLLKGVSQVQLQQPGAEVLVKPGASVKMSCKASGYTFTSYNMHWVKQTPG  
RGLEWIGAIYPNGDTSYNQFKKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYG  
GDWYFNVWGAGTTTVSAASQPRAHRSSPWHPPRAPLGAQRPWAAWSRTTSPNREPKSCD  
KTHTCPPCPMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV  
SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGGTRGVRGPHGQRPARPTLCPESD  
RCTNLCP TGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESENQOPENNYKT  
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSVHMHEALHNHYTQKSLSLSPGKSAEEAAAK  
EAAAKACDLPQTHNLRNKRALTLLVQMRLSPLSCLKDRKDFGFPQEKVDAQQIKKAQAI  
VLSLETQQILNIFTSKDSSAAWNATLLDSFCNDLHQQLNDLQGCLMQQVGVQEFLTQEDA  
LLAVRKYFHRTVYLREKKHSPCAWEVVRAEVWRALSSANVLGRLREEK

***Fig. 1H***

**αCD20-IgG1-huIFNα Gly<sub>4</sub>Ser linker - nucleic acid sequence**

ATGTACTTGGGACTGAACCTGTAAATCATAGTTTCTCTTAAAGGTGTCCAGAGTCAGGTACAAC  
TGCAGCAGCCTGGGCTGAGCTGCTGAAGCCTGGGCTCACTGAAGATGTCTGCAAGGCTTCTGG  
CTACACATTACCAAGTTACAATATGCACGGTAAAACAGACACCTGGTGGCTGGGCTGGAATGGATT  
GGAGCTATTATCCCGAAATGGTGTAACTTCCTACAATCAGAAGTTCAAAGGCAAGGCCACATTGA  
CTGCAGACAAATCCCTCCAGCACAGCCTACATGCAGCTCAGCAGCCTGACATCTGAGGACTCTGGGT  
CTATTACTGTGCAAGATCGACTTACTACGGCGGTGACTGGTACTTCATAITGCTGGGGCGCAGGGACC  
ACGGTCACCGTCTCTGCAGCTAGCCAACCAAGGGCCATCGGTCTTCCCCCTGGCACCCCTCCCAA  
GAGCACCTCTGGGGCACAGCGCCCTGGCTGCCCTGGTCAAGGACTACTTCCCCGAACCGGGAGCC  
CAAATCTTGACAAACTCACACATGCCAACCGTGGCCAAATGATCTCCCGAACCCCTGAGGTACAA  
TCCGTGGTGGTGGACGTGAGCCACGAAGACCTGAGGTCAAGTTCAAATGGTACGTGGACGGCGTGG  
AGGTGCATAATGCCAACGACAAAGCCGGGGAGGGAGCAGTACAACAGCACGTACCGGGTGGTCAAGCGT  
CCTCACCGTCTGCACCAAGGACTGGCTGAATGGCAAGGAGTACAAGTGAAGGTCTCCAACAAAGCC  
CTCCCAGCCCCATCGAGAAAACCATCTCCAAAGCCTAAAGGTGGGACCGTGGGGTGCAGGGCCAC  
ATGGACAGAGGCCGGCTGGCCCACCCCTGCTGCCCTGAGGTGACCGCTGTACCAACCTCTGCTC  
AGGGCAGCCCCGAGAACACAGGTGACACCCCTGCCCATCCGGGATGAGOTGAAAGAACCAACAG  
GTCAGCCTGACCTGCCCTGGTCAAAGGCTCTATCCAGCAGCATGCCGTGGAGTGGGAGAGCAATG  
GGCAGCCGGAGAACAACTACAAGACCAAGCCTCCGCTGCTGGACTCCGACGGCTCCTCTTCT  
CAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAAGCTTCTCATGCTCCGTGATGCAT  
GAGGCTCTGCACAAACCACTACACCGAGAACAGGCTCTCCCTGTCTCCGGTAATCTGGTGGCGGTG  
GATCCCTGTGATCTGCCCTCAAACCCACAGCCTGGTAGCAGGAGGACCTTGAATGCCCTGCCACAGAT  
GAGGAGAATCTCTCTTCTCCCTGCTGCTGAAGGACAGACATGACTTGGATTCCCCAGGAGGAGTT  
GGCAACCAGTTCAAAGGCTGAAACATCCCTGTCTCCATGAGATGATCCAGUAGATCTICAATC  
TCTTCAGCACAAAGGACTCATCTGCTGCTGGGATGAGACCCCTCTAGACAAATCTACACTGAAC  
CTACCAGCAGCTGAATGACTGGAAAGCCCTGTGTCAGATAACAGGGGTGGGGTGAACAGAGACTCCC  
ATGAAGGAGGACTCCATTCTGGCTGTGAGGAAATACTTCCAAAGAATGACTCTCTATCTGAAGAGA  
AGAAATACAGCCCTGTGCTGGAGCTGTCAGAGCAGAAATCATGAGATCTTTCTTGTCAAC  
AAACTTGCAAGAAAGITTAAGAAGTAAGGAATGA

**αCD20-IgG1-huIFNα Gly<sub>4</sub>Ser linker - amino acid sequence**

MYLGLNCVIIIVFLKGQSQVQLQQPGAEVLKPGASVKMSCKASGYTFTSYNMHWVKQTPG  
RGLEWIGAIYPNGDTSYNQKFKGKATLTADKSSTAYMQLSSLTSEDSAVYYCARSTYYG  
GDWYFNVWGAGTTTVSAASQPRAHRSSPWHPPPRAPLGAQRPWAAWSRTTSPNREPKSCD  
KTHTCPYCPMISRTPEVTCVVVDVSHEDPEVKFNWYVGVEVNNAKTKPREEQYNSTYRVV  
SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGGTRGVRGPHGQRPARPTLCPESD  
RCTNLCPYQPREPVYTLPPSRDELTKNQVSLTCLVKFYPSDIAVEWESENQOPENNYKT  
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCVMHEALHNHTQKSLSLSPGKSGGGSC  
DLPQTHSLGSRRTLMLLAQMRRISLFSLKDRHDFGFPQEEFGNQFQKAETIPVLHEMIQQ  
IFNLFSTKDSSAAWDETLLDKFYTELYQQLNDLEACVIQGVGVTEPLMKEDSILAVRKYF  
QRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLRSKE

***Fig. 11***

**αCD20-IgG1-huIFNα alpha helical linker - nucleic acid sequence**

ATGTACTTGGGACTGAACGTGTAACTCATAGTTTTCTCTTAAAAGGTGTCCAGAGTCAGGTACAAC  
 TGCAGCAGCCTGGGGCTGAGCTGGTGAAGCCTGGGCGCTCAGTGAACAGTGTCTGCAAGGCTCTGG  
 CTACACATTIACCACTTACAATATGCACCTGGTAAAACAGACACCTGGTGGGGCTGGATGGATT  
 GGAGCTATTTATCCCGGAAATGGTGTAACTTCTACAATCAGAAGTCAAAGGCAAGGCCACATTGA  
 CTGGAGACAAATCCTCCAGCACAGCCTACATGCAGCTCAGCAGCCTGACATCTGAGGACTCTGGGGT  
 CTATTACTGTGCAAGATCGACTTACTACGGCGGTGACTGTACTTCAATGCTGGGCGAGGGACC  
 ACGGTACCCGTCTGCAAGCTAGCCAACCAAGGGGCCATCGGTCTTCCCCCTGGCACCCCTCTCCAA  
 GAGCACCTCTGGGGCACAGCGGCCCTGGCTGGTCAAGGACTACTTCCCCGAACCGGGGASCC  
 CAAATCTTGTGACAAAACACACATGCCAACCGTGCCCCAATGATCTCCCGGACCOCTGAGGTCACA  
 TGGCTGGTGGGACGTGAGCCACGAAGACCCGTAGGTCAAGTTCAACTGGTACGTGGACGGCGTGG  
 AGGTGCAATAATGCCAAGACAAAGGCGGGAGGGAGCAGTACAACAGCACGTACCGGGTGGTCAAGCGT  
 CCTCACCGTCTGCAACCAAGGACTGGCTGAATGCAAGGAGTACAAGTGTCAAGGTCTCCAACAAAGCC  
 CTCCCAGCCCCATCGAGAAAACCATCTCAAAGCCAAGGTGGGACCCGTGGGGTGGAGGGGCOAC  
 ATGGACAGAGGCCGGCTGGCCCACCCCTGAGAGTGGCCGTGTACCAACCTCTGTCTAC  
 AGGGCAGCCCCGAGAACCAACAGGTGACACCCCTGCCCATCCGGATGAGCTGACCAAGAACAG  
 GTCAAGCTGACCTGCCTGGTCAAAGGCTCTATCCACGGACATGCCGTGGACTCCGACGGCTCTTCTCTA  
 CAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTCTCATGCTCCGTGATGCAT  
 GAGGCTCTGACAAACCAACTACACCGAGAAGAGCCTCTCTGTGATCTGCCTCAAACCCACAGCCTGGTAGCAGGAG  
 CTAAGAGGCCAGCCAAGCGGGATCCTGTGATCTGCCTCAAACCCACAGCCTGGTAGCAGGAG  
 GACCTTGATGCTCTGGCACAGATGAGGAGAATCTCTCTCTCTGTGAGGACAGACATGAC  
 TTGGATTTCCCCAGGAAGAGTTGGCAACCAGTTCCAAAAGGCTGAAACCATCCCTGTCTCCATG  
 AGATGATCCAGCAGATCTCAATCTCTCAGCACAAAGGACTCATCTGCTGCTGGATGAGACCT  
 OCTAGACAAATTCTACACTGAACCTACAGAGCTGAATGACCTGGAAGCCTGTGAGGAAATCTTCAA  
 GTGGGGGTGACAGAGACTCCCTGATGAGGAGGACTCCATTCTGGCTGTGAGGAAATCTTCAA  
 GAATCACTCTCTATCTGAAAGAGAAATACAGCCCTGTGCCTGGAGGTGTCAGAGCAGAAAT  
 CATGAGATCTTTCTTGTCAACAAACTTGCAAGAAAGTTAAGAAGTAAGGAATGA

**αCD20-IgG1-huIFNα alpha helical linker - amino acid sequence**

MYLGLNCVIIVFLLKGVQSQVQLQQPAGAEELVKPGASVKMSCKASGYTFTSYNMHWVKQTPG  
 RGLEWIGAIYPGNQDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYG  
 GDWYFNVWGAGTTVTCAASQPKAHRSSPWHPPPAPLGAQRPWAWSRTTSPNREPSCD  
 KTHICPPCPMISRTPEVTCVVVDVSHDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV  
 SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGGTRGVRGPHGQRPAPPTLCPSED  
 RCTNLCP TGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAYEWESNGQOPENNYKT  
 TFPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKA**EEAAAKE**  
**AAAKAGSCDLQTHSILGSRRTLMLLAQMRRISLFSCLKDRHDFGFPQEFGNQFQKAETIP**  
 VLHEMIQQIENLFSTKQSSAAWDETLLOKFYTELYQQLNDLEACVIQGVGVTEPLMKEDS  
 ILAVRKYFQRITLYLKEKKYSPCAEVVRAEIMRSFSLSTNLQESLRSE

***Fig. 1J***

**αHer2/neu light chain - nucleic acid sequence**

ATGGGATGGAGCTGGGTAAATCCTCTTCTCCTGTCAGTAAC TGCAGGTGTCCACTCCCAGT  
CTGTGTTGACGCCAGCGCCCTCAGTGTCTGCGGCCAGGACAGAAGGTACCATCTCCTG  
CTCTGGAAGCAGCTCCAACATTGGAATAATTATGTATCCTGGTACCAGCAGCTCCAGGA  
ACAGCCCCAAACTCCTCATCTATGATCACACCAATCGGCCGCAGGGGTCCTGACCGAT  
TCTCTGGCTCCAAGTCTGGCACCTCAGCCTCCCTGGCCATCAGTGGGTTCCGGTCCGAGGA  
TGAGGCTGATTATTACTGTGCCTCCTGGACTACACCCCTCTCGGCTGGGTGTTGGAGGA  
GGGACCAAGGTACCGTCCTAGGTGAG

**αHer2/neu light chain - Amino acid sequence**

MGWSWVILFLLSVTAGVHSQSVLTQPPSVSAAPGQKVTISCSGSSNIGNNYVSWYQLPG  
TAPKLLIYDHTNRPAGVPDRFSGSKSGTSASLAISGFRSEDEADYYCASWDYTL SGWVF GG  
GTKVTVLGE

***Fig. 1K***

**αHer2/neu-IgG1-muIFNα glyser linker - Nucleic acid sequence**

ATGGGATGGAGCTGGGTAAATGCATCTTCTCCTGTCAGTAAC TGCA GATGCCCGGGAAAGGCCTGGA  
GTACATGGGCTCATCTATCCTGGTACTCTGACACCAAATACAGCCGTCCTCCAAGGCCAGGTC  
ACCATCTCAGTCGACAAGTCCGTAGCACTGCCTACTTGCATGGAGCAGTCTGAAGCCCTCGGACA  
GCGCCGTGTATTTGTGCGAGACATGACGTGGGATATTGACCCGACCGGACTTGCACAAAGTGGCC  
TGAATACTTCCAGCATTGGGCCAGGGCACCTGGTCACCGTCTCAGCTAGCCAACCAAGGGCC  
CATCGGTCTTCCCCCTGGCACCCCTCCAAGAGCACCTCTGGGGCACAGCGCCCTGGGCTGCCT  
GGTCAAGGACTACTTCCCCGAACCGGGAGCCAAATCTTGTGACAAAACACTCACACATGCCAACCGTG  
CCCAATGATCTCCGGACCCCTGAGGTACATGCGTGGTGGACGTGAGCCACGAAGACCCCTGAG  
GTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGC  
AGTACAACAGCACGTACGGGTGGTCAGCGTCTCACCGTCTGCACCAGGACTGGCTGAATGGCAA  
GGAGTACAAGTGAAGGTCTCCAACAAAGCCCTCCCAGCCCCATCGAGAAAACCATCTCCAAGCC  
AAAGGTGGGACCCGTGGGGTGCAGGGCACATGGACAGAGGCCGGCTGGCCACCCCTGCC  
AGAGTGACCGCTGTACCAACCTCTGCTTACAGGGCAGCCCCGAGAACCCACAGGTGTACACCCCTGCC  
CCCATCCCAGGATGAGCTGACCAAGAACCCAGGTCAAGCTGACCTGCTGGTCAAAGGCTTATCCC  
AGCGACATGCCGTGGAGTGGAGAGCAATGGCAGCCGGAGAACAACTACAAGACCACGCCCTCCG  
TGCTGGACTCCGACGGCTCTTCTTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCA  
GGGGAACGTCTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCAACTACACGCAGAACAGCCTC  
TCCCTGTCTCCGGTAAATCTGGTGGCGGTGGATCTGACCTGCTCAGACTCATAACCTCAGGA  
ACAAGAGAGCCTTGACACTCTGGTACAAATGAGGAGACTCTCCCTCTCCTGCTGAAGGACAG  
GAAGGACTTTGGATTCCCGCAGGAGAACGGTGGATGCCAGCAGATCAAGAAGGCTCAAGCCATCCCT  
GTCCTGAGTGAGCTGACCCAGCAGATCTGAACATCTTACATCAAAGGACTCATCTGCTGCTTGG  
ATGCAACCCCTCTAGACTATTCTGCAATGACTCCACCAGCAGCTCAATGACTGCAAGGTTGTCT  
GATGCAGCAGGTGGGGTGCAGGAATTCCCTGACCCAGGAAGATGCCCTGCTGGCTGTGAGGAAA  
TACTTCCACAGGATCACTGTGTACCTGAGAGAGAACACAGCCCTGTGCTGGAGGTGGTCA  
GAGCAGAAGTCTGGAGAGCCCTGCTTCCCTGCCAATGTGCTGGAAAGACTGAGAGAACAGAGAAATG  
A

**αHer2/neu-IgG1-muIFNα glyser linker - Amino acid sequence**

MGSWVVMHLSPVSNCMPKGKLEYMGLIYPGDSDTKYSPSFQGVTLISVDKSVSTAYLQWSS  
LKP SDSAVYFCARHDVGYCDRTCAKWEYFQHWGQGTIVTSSASQPRAHRSSPWHPPR  
APLGAQRPWAAWSRTTSPNREPKSCKDTHCPCPMISRPEVTCVVVDVSHEDPEVKFNW  
YVDGVEVHNAKTPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK  
AKGGTRGVRGPHQRPARPTLCPESDRCTNLCPTGQPREPQVYTLPPSRDELTKNQVSLTC  
LVKGFPYPSDIAVEWE SNGQPENNYKTPPVLDSDGSFFFLYSKLTVDKSRWQQGNVFSCSVM  
HEALHNHYTQKSLSPGKSGGGGSCDLPQTHNLRNKALTLLVQMRRILSPLSCLKDRKDF  
GFPQEKVDAQQIKKAQAIPLSELTQQILNIFTSKDSAAWNATLLDSFCNDLHQQLNDLQ  
GCLMQQVGVQEFPLTQEDALLAVRKYFHRI TVYLREKKHSPCAWEVVRAEVWRALSSANV  
LGRLREEK

***Fig. 1L***

**$\alpha$ Her2/neu-IgG1-muIFN $\alpha$  alpha helical linker - nucleic acid sequence**

ATGGGATGGAGCTGGGTAAATGCATCTTCTCTGTCACTAAGTCAGCAGATGCCCGGGAAAGGCCTGGA  
GTACATGGGCTCATCTATCCTGGTACTCTGACACCAAATACAGCCCCTCCAAGGCCAGGTC  
ACCATCTCAGTCGACAAGTCCGTCACTGCCTACTTGCATGGAGCAGTCTGAAGCCCTCGGACA  
GCGCCGTGTATTTGTGCGAGACATGACGTGGATATTGCACCGACCGACTTGCCTGCAAAGTGGCC  
TGAATACTTCCAGCATTGGGCCAGGGCACCCCTGGTCAACCGTCTCCTCAGCTAGCCAACCAAGGGCC  
CATCGGTCTTCCCCCTGGCACCCCTCCAAGAGCACCTCTGGGCCACAGCGGCCCTGGCTGCCT  
GGTCAAGGACTACTTCCCCGAACCGGGAGCCCAAATCTTGTGACAAAACACACATGCCAACCGTG  
CCCAATGATCTCCGGACCCCTGAGGTACATGCGTGGTGGACGTGAGCCACGAAGAACCTGAG  
GTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGGGAGGAGC  
AGTACAACAGCACGTACGGGTGGTCAGCGTCCTCACCGTCTGCACCAGGACTGGCTGAATGGCAA  
GGAGTACAAGTGAAGGTCTCCAACAAAGCCCTCCCAGCCCCATCGAGAAAACCATCTCAAAGCC  
AAAGGTGGGACCGTGGGTGCGAGGGCCACATGGACAGAGGCCGCTGGCCACCCCTGCCC  
AGAGTGACCGCTGTACCAACCTCTGCCTACAGGGCAGCCCCGAGAACACAGGTGTACACCCCTGCC  
CCCATCCCAGGATGAGCTGACCAAGAACCCAGGTCAGCCTGACCTGCTGGTCAAAGGCTCTATCCC  
AGCGACATCGCCGTGGAGTGGAGAGCAATGGCAGCCGGAGAACAAACTACAAGACCACGCCCTCCG  
TGCTGGACTCCGACGGCTCTTCTTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCA  
GGGGAAACGTCTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCAACTACACGAGAACGCTC  
TCCCTGTCTCCGGTAAAGCAGAGGCCGAGCTAAAGAGGCCGAGCCAAAGCAGGATCTGTGACC  
TGCCTCAGACTCATAACCTCAGGAACAAGAGAGCCTGACACTCTGGTACAAATGAGGAGACTCTC  
CCCTCTCTGCCTGAAGGACAGGAAGGACTTGGATTCCCGAGGAGAACGGATGCCAGCAG  
ATCAAGAAGGCTCAAGCCATCCCTGCTGAGTGAGCTGACCCAGCAGATCCTGAACATCTCACAT  
CAAAGGACTCATGCTGTTGGAATGCAACCCCTCTAGACTCATTCTGCAATGACCTCACCAGCA  
GCTCAATGACCTGCAAGGTTGTCTGATGCAGCAGGTGGGGTGCAGGAATTCCCTGACCCAGGAA  
GATGCCCTGCTGGCTGTGAGGAAATACTCCACAGGATCACTGTGACCTGAGAGAGAACACA  
GCCCTGTGCTGGAGGTGGTCAGAGCAGAAGTCTGGAGAGCCCTGTCTCCCTGCCAATGTGCT  
GGGAAGACTGAGAGAACAGAACATGA

 **$\alpha$ Her2/neu-IgG1-muIFN $\alpha$  alpha helical linker - amino acid sequence**

MGWSWVMHLSPVSNCMPGKLEYMGLIYPGDSDTKYSPSFQGQVTISVDKSVSTAYLQWSS  
LKP SDSAVYFCARHDVGYCDRTCAKWPEYFQHWGQGTLVTSSASQPRAHRSSPWHPPPR  
APLGAQRPWAAWSRTTSPNREPKSCKDKHTCPPCPMISRTPEVTCVVVDVSHEDPEVKFNW  
YVDGVEVHNAKTPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK  
AKGGTRGVRGPHQRPARPTLCPESDRCTNLCPGQPREPVYTLPPSRDELTKNQVSLTC  
LVKGFPYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM  
HEALHNHYTQKSLSLSPGKSAEAAAKEAAKACDLPQTHNLRNKRALLLVQMRRRLSPLSC  
LKDRKDFGFPQEKFVDAQQIKKAQAIPLSELTQQILNIFTSKDSSAAWNATLLDSFCNDLH  
QQLNLDLQGCLMQQVGVQEFPKTQEDALLAVRKYFHRITVYLREKKHSPCAWEVVRAEVWRA  
LSSSANVLGRLREEK

***Fig. 1M***

**αHer2/neu-IgG1-huIFNα glyser linker - nucleic acid sequence**

ATGGGATGGAGCTGGTAATGCATCTTCTCTGTCACTGCAGATGCCGGAAAGGCCTGGA  
GTACATGGGCTCATCTACCTGGTACTCTGACACCAAATACAGCCGTCTCCAAGGCCAGGTC  
ACCATCTCAGTCAGAAGTCCGTAGCACTGCCTACTTGCATGGAGCAGTCTGAAGCCCTCGGACA  
GCGCCGTGTATTGGCGAGACATGACGTGGGATATTGCACCGACCGACTGCGCAAAGTGGCC  
TGAATACTTCCAGCATTGGGCCAGGGCACCTGGTACCGTCTCAGCTAGCCAACCAAGGGCC  
CATCGGTCTTCCCCCTGGCACCCCTCCAAGAGCACCTCTGGGGCACAGCGCCCTGGCTGCCT  
GGTCAAGGACTACTTCCCCAACCGGGAGCCAAATCTTGTGACAAAACACACATGCCACCCTG  
CCCAATGATCTCCGGACCCCTGAGGTACATGCGTGGTGGACGTGAGCCACGAAGACCCCTGAG  
GTCAAGTTCAACTGGTACGTGGACGGGTGGTACGCGTCTCACCCTGCACCAGGACTGGCTGAATGGCAA  
AGTACAACAGCACGTACCGGGTGGTACGCGTCTCACCCTGCACCAGGACTGGCTGAATGGCAA  
GGAGTACAAGTGCAGGTCTCAACAAAGCCCTCCCAGCCCCATCGAGAAAACCATCTCAAAGCC  
AAAGGTGGGACCCGTGGGTGCGAGGGCCACATGGACAGAGGCCGGCTGGCCACCCCTGCC  
AGAGTGACCGCTGTACCAACCTCTGCCTACAGGGCAGCCCCGAGAACACAGGTGTACACCCTGCC  
CCCATCCGGGATGAGCTGACCAAGAACCAAGGTCAGCTGACCTGCTGGTCAAAGGCTTATCCC  
AGCGACATGCCGTGGAGTGGAGAGCAATGGCAGCCGGAGAACAAACTACAAGACCACGCCCTCCG  
TGCTGGACTCCGACGGCTCCTTCTTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCA  
GGGAACGTCTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAACAGCCTC  
TCCCTGCTCCGGTAAATCTGGTGGCGGTGGATCCTGTGATCTGCTCAAACCCACAGCCTGGTA  
GCAGGAGGACCTGATGCTCCTGGCACAGATGAGGAGAACCTCTCTTCTCTGGTGAAGGACAG  
ACATGACTTGGATTCCCCAGGAGGAGTTGGCAACCAGTCCAAAAGGCTGAAACCACCCCTGTC  
CTCCATGAGATGATCCAGCAGATCTCAATCTTCAGCACAAAGGACTCATCTGCTGCTGGATG  
AGACCCTCCTAGACAAATTCTACACTGAACCTACAGCAGCTGAATGACCTGGAAGCCTGTTGAT  
ACAGGGGGTGGGGGTGACAGAGACTCCCTGATGAAGGGAGACTCCATTCTGGCTGTGAGGAATAC  
TTCCAAAGAATCACTCTATCTGAAGAGAAGAAATACAGCCCTGTGCCTGGAGGTTGTCAGAG  
CAGAAATCATGAGATCTTTCTTGCAACAAACTGCAAGAAAGTTAAGAAGTAAGGAATGA

**αHer2/neu-IgG1-huIFNα glyser linker - nucleic acid sequence**

MYLGLNCVIIVFLLKGVSQVQLQQPGAEVLVKPGASVKMSCKASGYFTSYNMHWVKQTPG  
RGLEWIGAIYPNGDTSYNQFKKGATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYG  
GDWYFNVWGAGITVTVSAAQPRAHRSSPWHPPPAPLGAQRPWAAWSRTTSPNREPSCD  
KTHTCPPCPMSRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVNAKTKPREEQYNSTYRVV  
SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGGTRGVRGPHGQRPARPTLCPESD  
RCTNLCP TGQPREPVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT  
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKSGGGSC  
DLPQTHSLGSRRTLMLLAQMRRISLFSLKDRHDFGFPQEFGNQFQKAETIPVLHEMIQQ  
IFNLFSTKDSSAAWDETLLDKFYTELYQQLNDLEACVIQGVGVTEPLMKEDSILAVRKYF  
QRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLRSKE

***Fig. 1N***

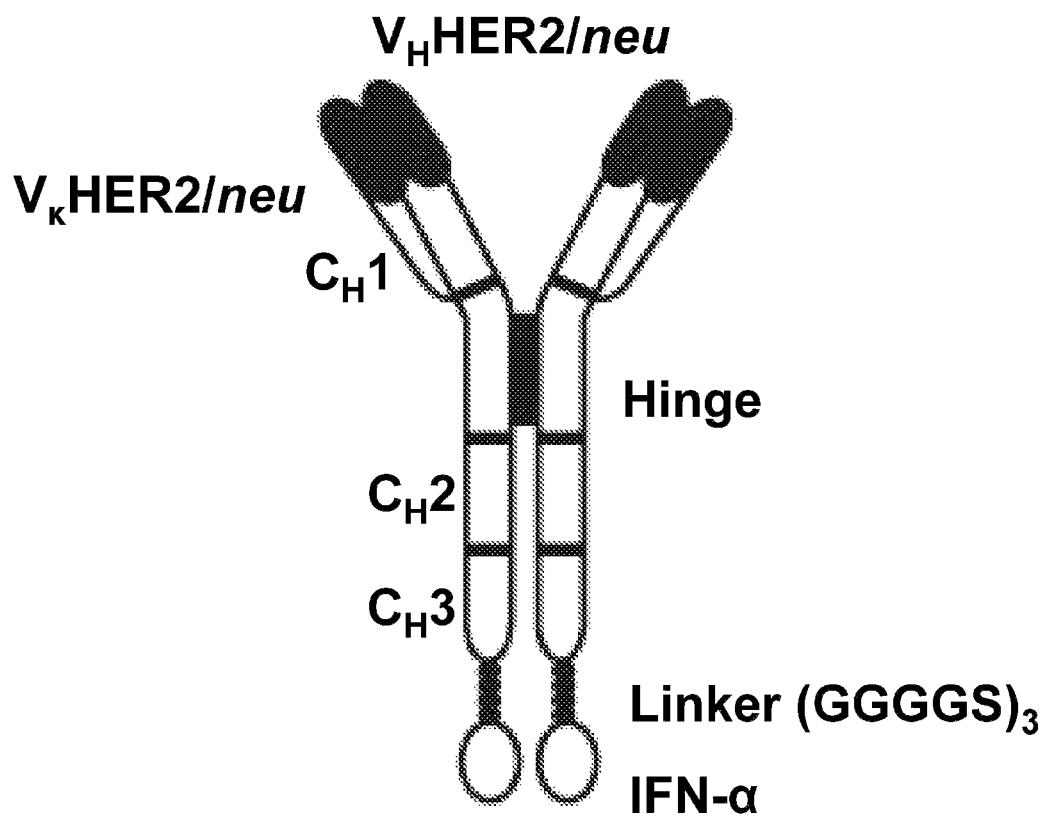
**aHer2/neu-IgG1-huIFN $\alpha$  alpha helical linker - nucleic acid sequence**

ATGGGATGGAGCTGGTAATGCATCTTCTCCTGTCACTGAGATGCCGGAAAGGCCTGGA  
GTACATGGGCTCATCTACCTGGTACTCTGACACCAAATACAGCCCCTCCAAGGCCAGGTC  
ACCATCTCAGTCGACAAGTCCGTAGCACTGCCACTTGCAATGGAGCAGTCTGAAGCCCTCGGACA  
GCGCCGTGATTGGTGCAGACATGACGTGGATATTGCACCGACGGACTTGCAGAAAGTGGCC  
TGAATACTCCAGCATTGGGCCAGGGCACCCCTGGTCACCGTCTCCTCAGCTAGCCAACCAAGGGCC  
CATCGGTCTCCCCCTGGCACCCCTCCAAGAGCACCTCTGGGGCACAGCGGCCCTGGCTGCCT  
GGTCAAGGACTACTTCCCCGAACCGGGAGCCAAATCTTGTGACAAAACATCACACATGCCAACCGTG  
CCCAATGATCTCCGGACCCCTGAGGTACATGCGTGGTGGACGTGAGCCACGAAGACCCGTAG  
GTCAAGTTCAACTGGTACGGACGGCTGGAGGTGCATAATGCCAAGACAAAGCCGGAGGAGC  
AGTACAACAGCACGTACGGGTGGTACGCTCCTCACCGTCTGCACCAGGACTGGCTGAATGGCAA  
GGAGTACAAGTGCAGGTCTCAAACAAAGCCCTCCCAGCCCCATCGAGAAAACCATCTCAAAGCC  
AAAGGTGGGACCCGTGGGTGCGAGGGCACATGGACAGAGGCCGCTGGCCACCCCTGCCCTG  
AGAGTGACCGCTGTACCAACCTCTGCCTACAGGGCAGCCCCGAGAACACCAGGTGTACACCCTGCC  
CCCATCCGGATGAGCTGACCAAGAACCCAGGTACGCTGACCTGCCTGGTCAAAGGCTTCTATCCC  
AGCGACATGCCGTGGAGTGGAGAGCAATGGCAGCCGAGAACAACTACAAGACCACGCCCTCCC  
TGCTGGACTCCGACGGCTCTTCTTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCA  
GGGGAACGTCTCTCATGCTCGTGTGATGCATGAGGCTGACAAACCACTACACGCAGAACAGCCTC  
TCCCTGTCTCCGGTAAAGCAGAGGCCGAGCTAAAGAGGCCGAGCCAAAGCGGGATCTGTGATC  
TGCCTCAAACCCACAGCCTGGTAGCAGGAGACCTGACTTGGATTCCCCAGGAGGAGTTGGCAACCAGTTC  
CAAAAGGCTGAAACCATCCCTGCTCCATGAGATGATCCAGCAGATCTCAATCTTCAAGC  
AGGACTCATCTGCTGGATGAGACCCCTCTAGACAAATTCTACACTGAACCTACCCAGCAGCT  
GAATGACCTGGAAGCCTGTTGATACAGGGGTGGGGTGACAGAGACTCCCTGATGAAGGAGGAC  
TCCATTCTGGCTGTGAGGAAATACTCCAAAGAATCACTCTATCTGAAAGAGAAGAAATACAGCC  
CTTGTGCCTGGGAGGTTGTCAAGAGCAGAAATCATGAGATCTTTCTTGTCAACAAACTTGCAAGA  
AAGTTAAGAAGTAAGGAATGA

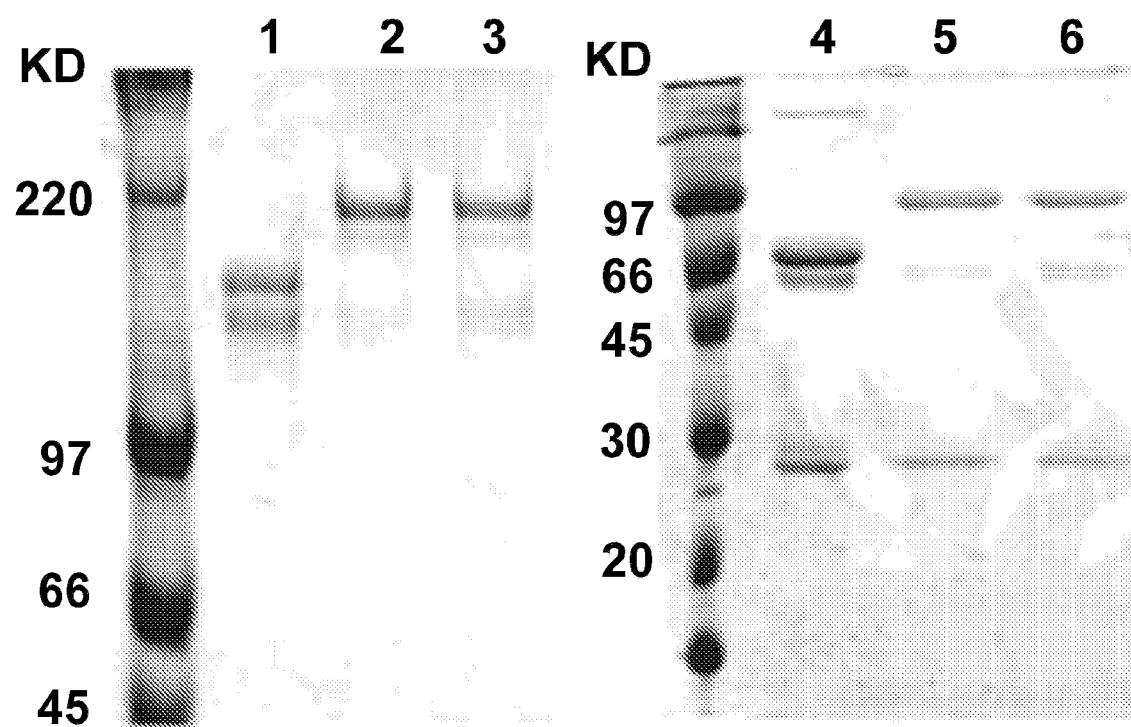
**aHer2/neu-IgG1-huIFN $\alpha$  alpha helical linker - amino acid sequence**

MYLGLNCVIIVFLLKGVQSQVQLQQPGAEVLVKPGASVKMSCKASGYTFTSYNMHWVKQTPG  
RGLEWIGAIYPNGDTSYNQKFKGKATLTADKSSTAYMQLSLTSED SAVYYCARSTYYG  
GDWYFNWGAGTTVTVAASQPRAHRSPPHPPRPLGAQRPWAAWSRTSPNREP KSCD  
KTHTCPPCPMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV  
SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIASKGGTRGVRGPHGQRPARPTLC PESD  
RCTNLCP TGQPREPVYTLPPSRDELTKNQVSLTCLVKGFYPSDI AVEWE SNGQ PENNYKT  
TPPVLDSDGSFFLYSKLTVDKSRWQQGNFSCSVMHEALHNHTQKSLSLSPGKSAEAAAK  
EAAAKACDLPQTHSLGSRRTLMLAQMRRI SLFSCLKDRHDFGPQEEFGNQFQKAETIPV  
LHEMIQQIFNLFDSSAAWDETLLDKFYTELYQLNDLEACVIQGVGVETPLMKEDSI  
LAVRKYFQRITLYLKEKKYSPCAEVVRAEIMRSFSLSTNLQESLR SKE

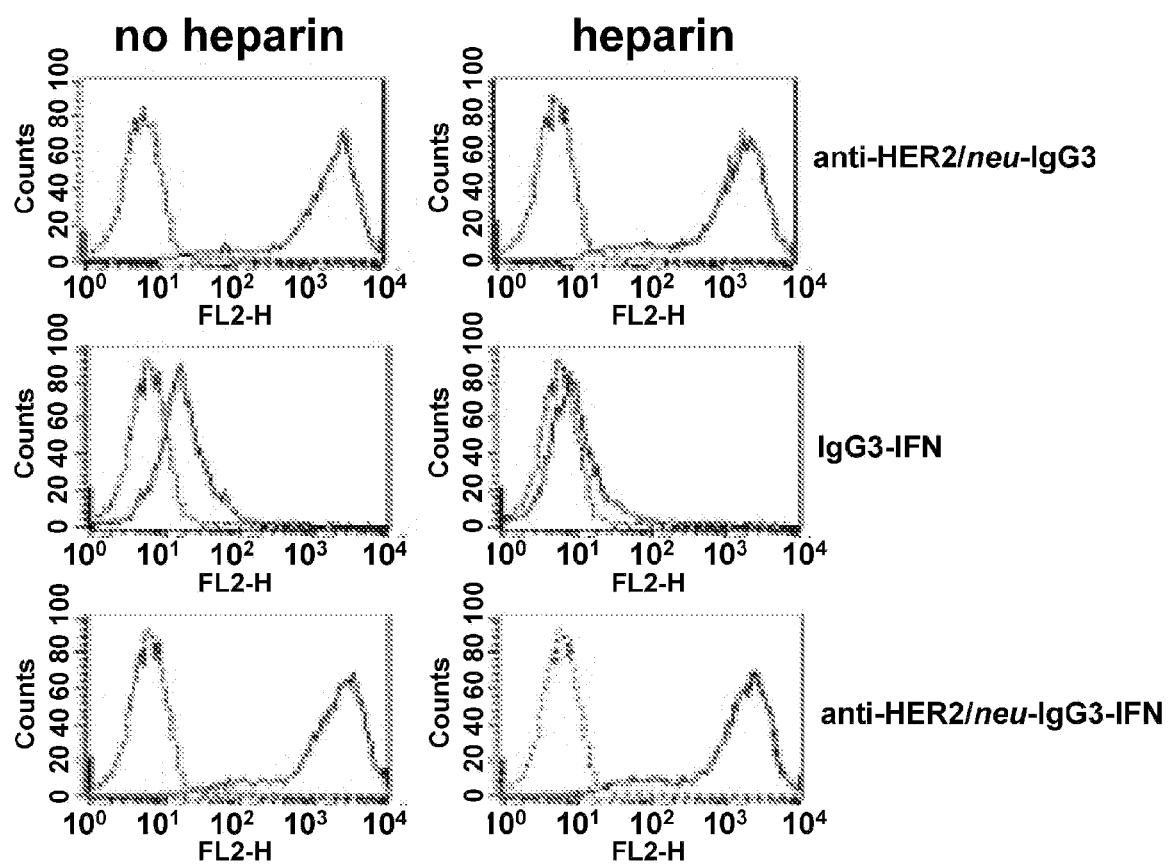
***Fig. 1o***



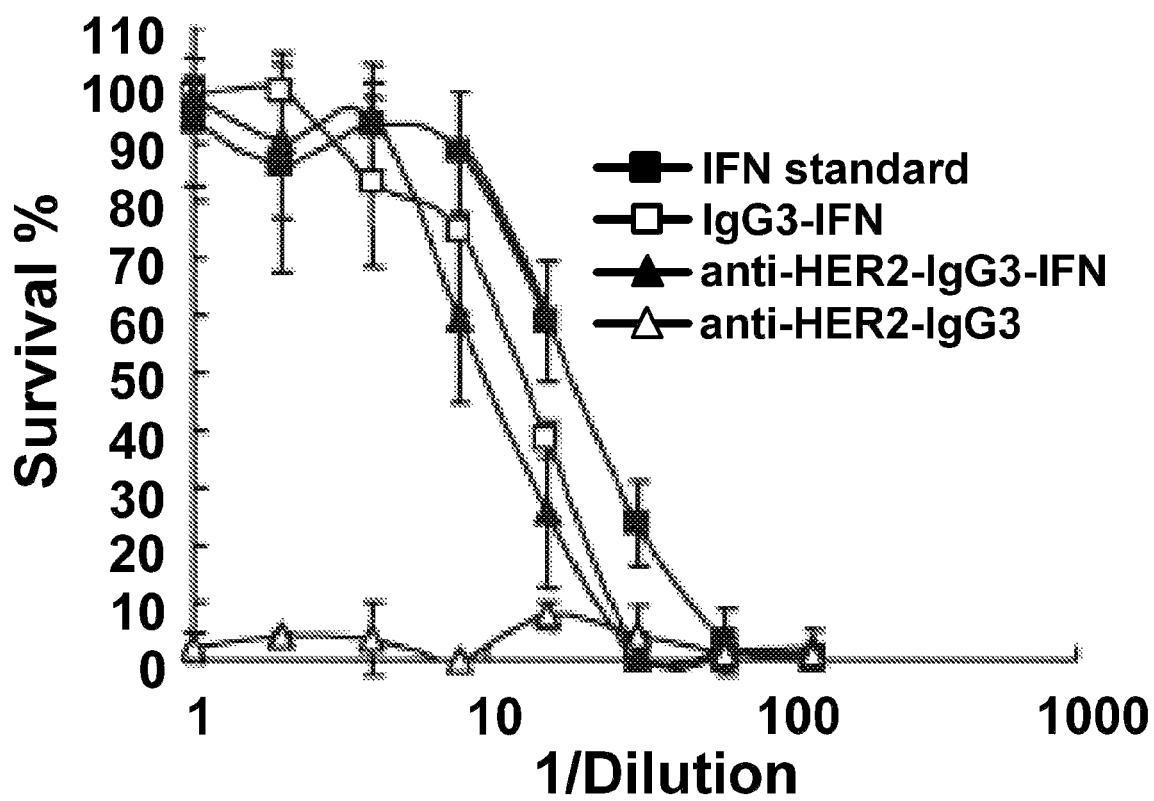
*Fig. 2A*



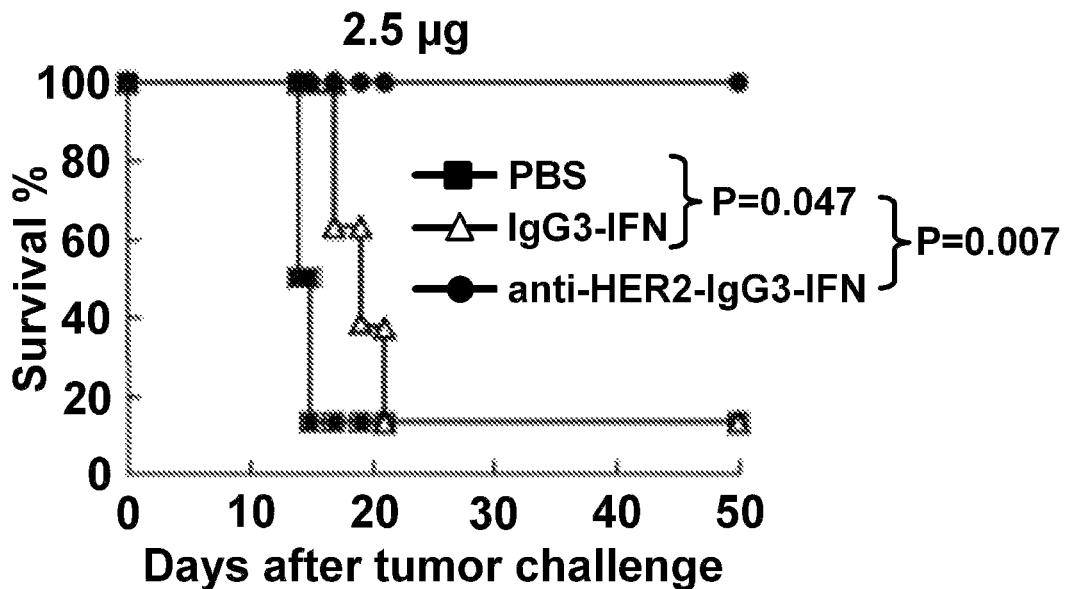
*Fig. 2B*



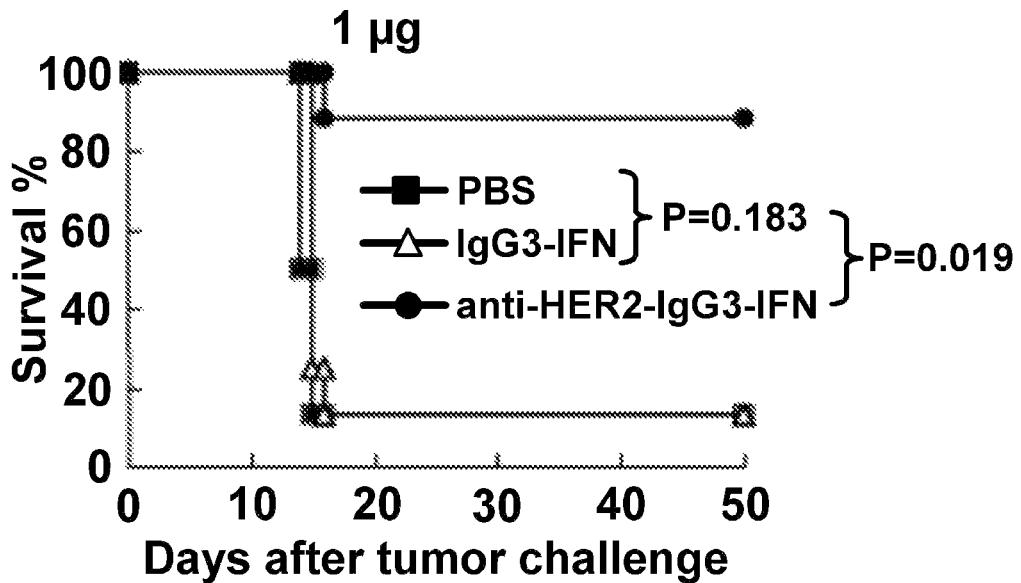
***Fig. 2C***



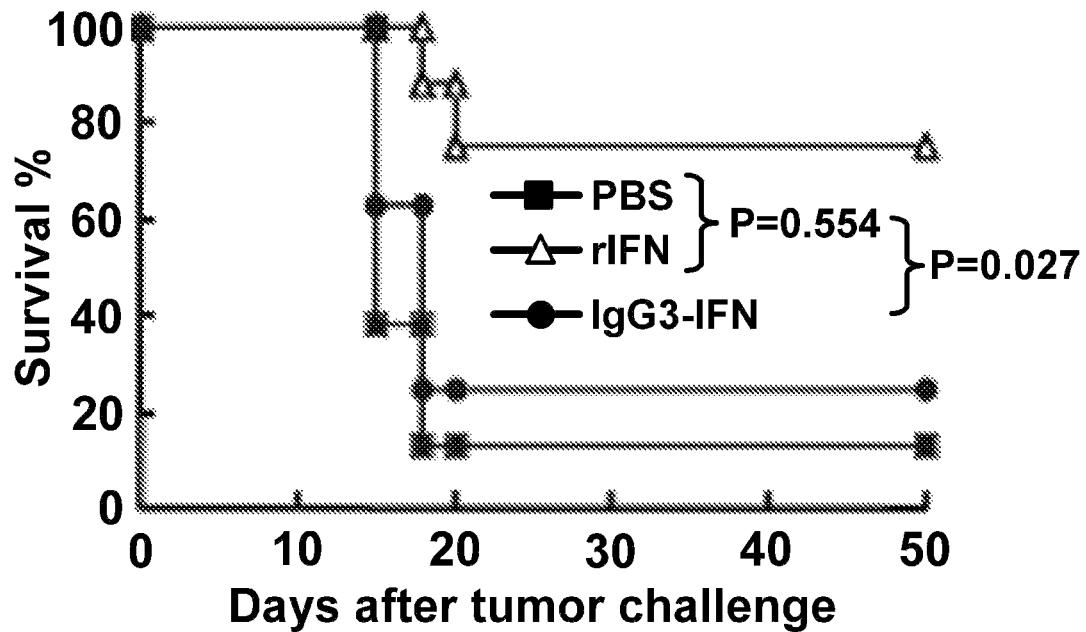
*Fig. 2D*



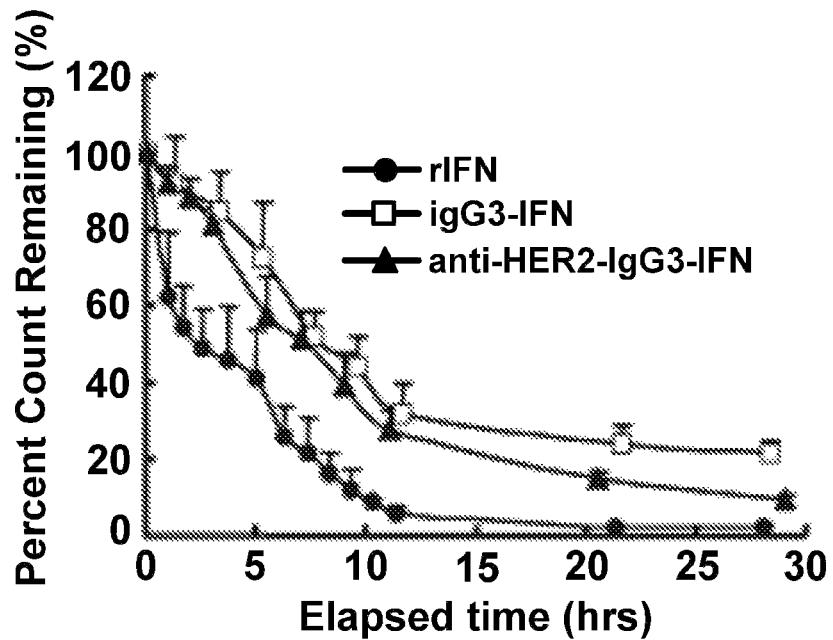
*Fig.3A*



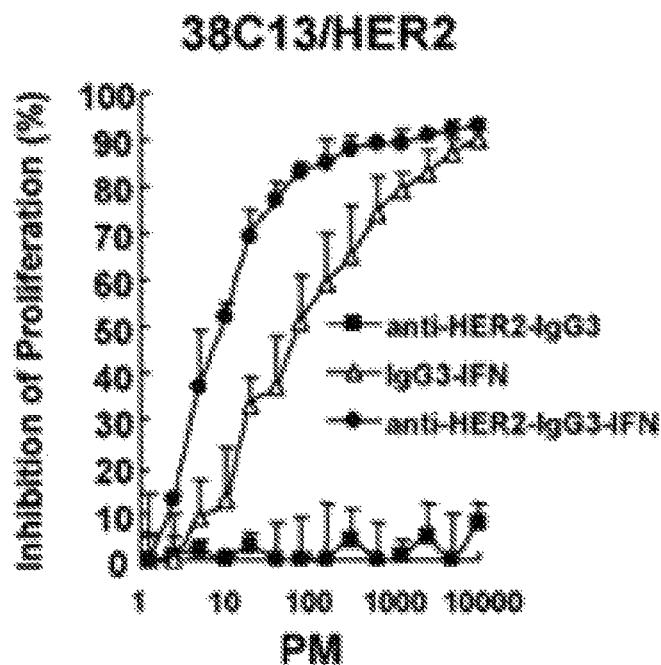
*Fig.3B*



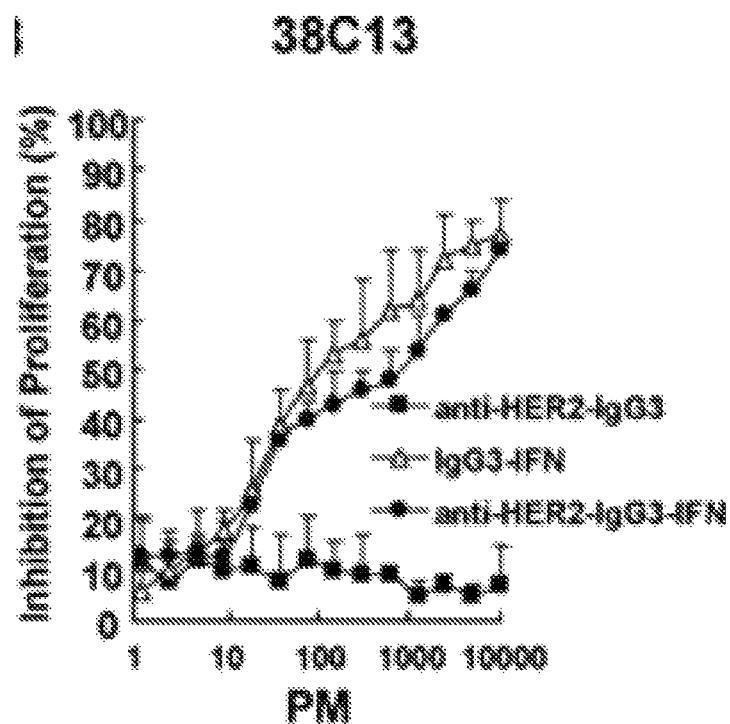
*Fig.4A*



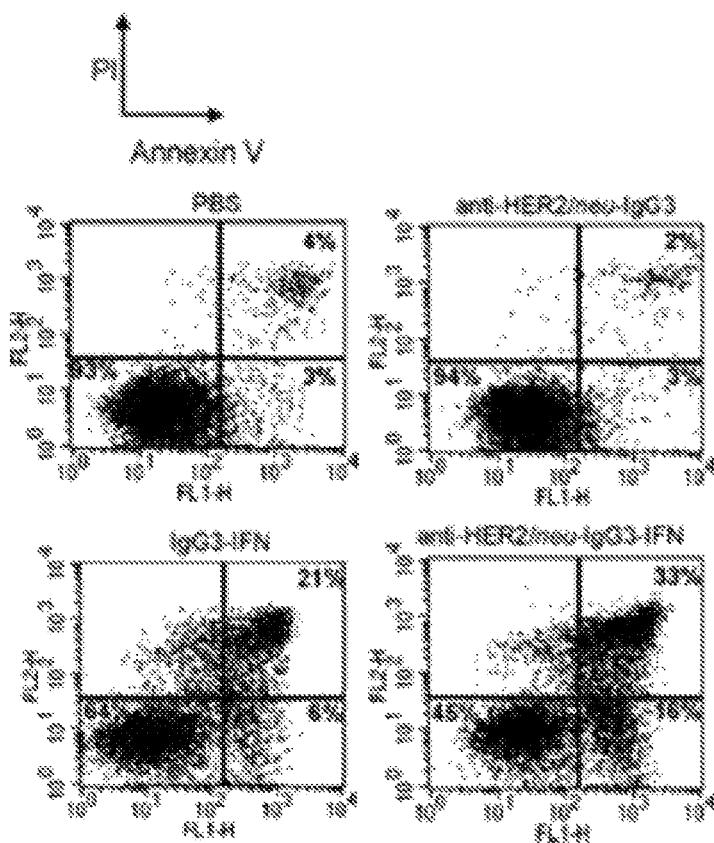
*Fig.4B*



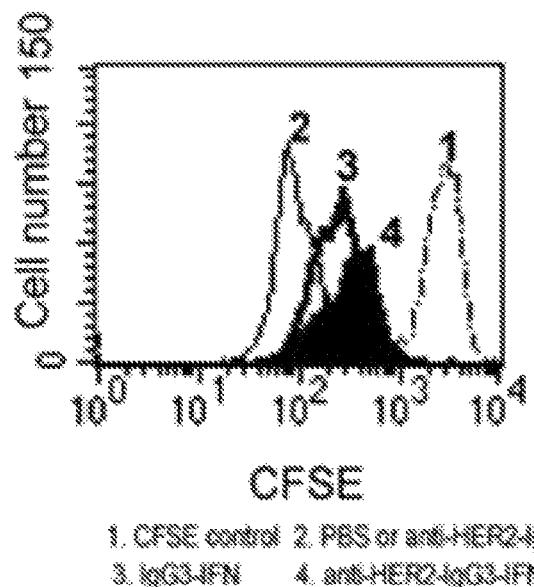
*Fig.5A*



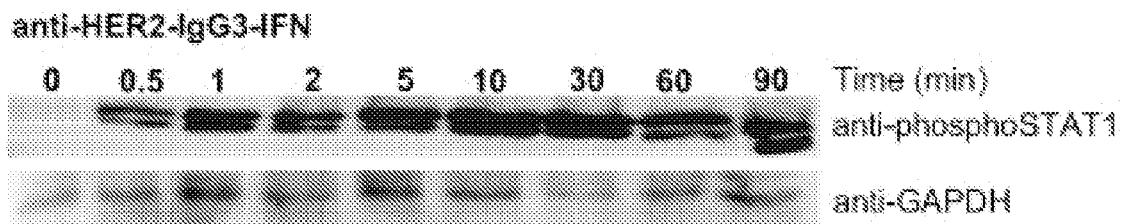
*Fig.5B*



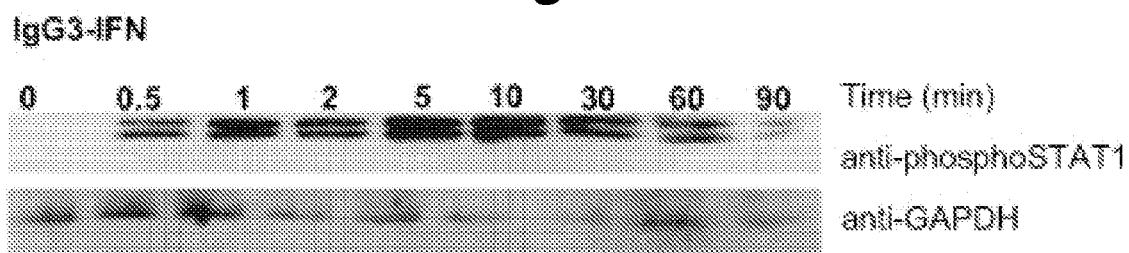
**Fig.5C**



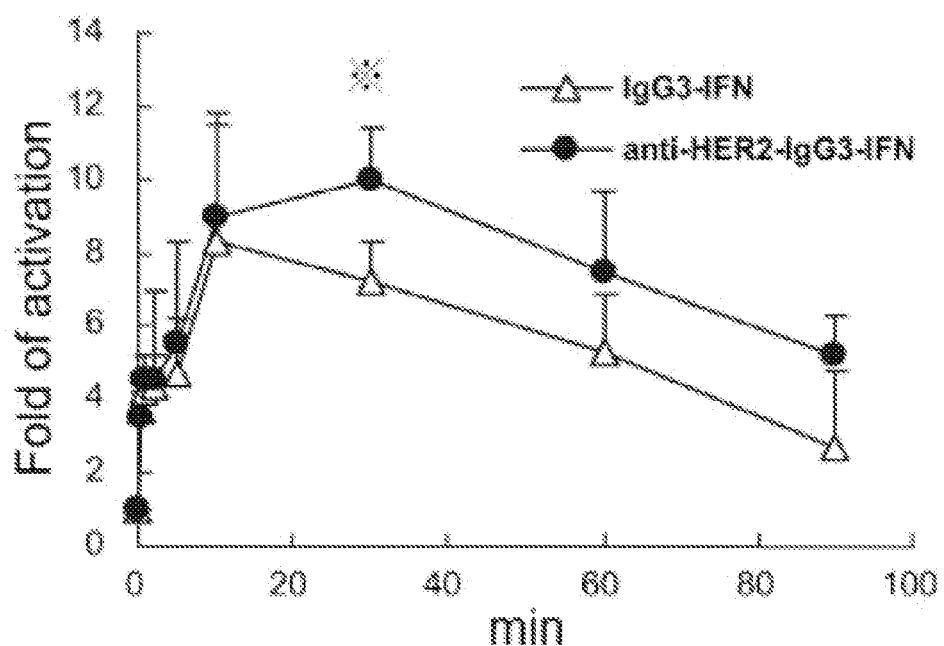
**Fig.5D**



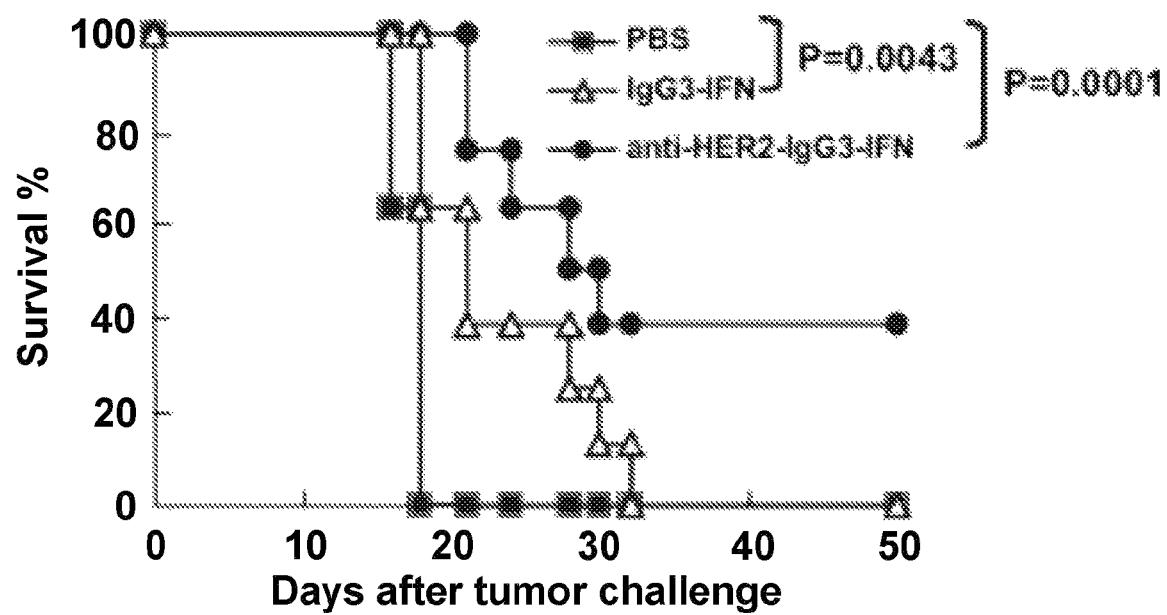
*Fig.6A*



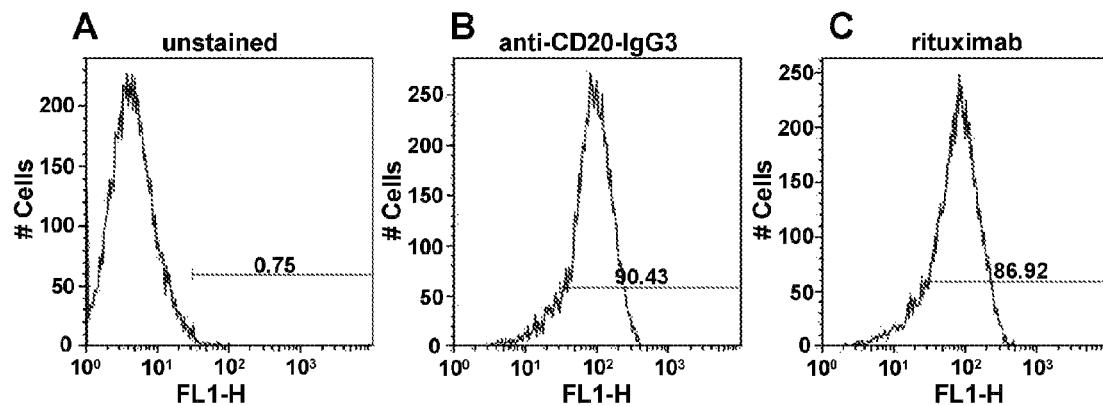
*Fig.6B*



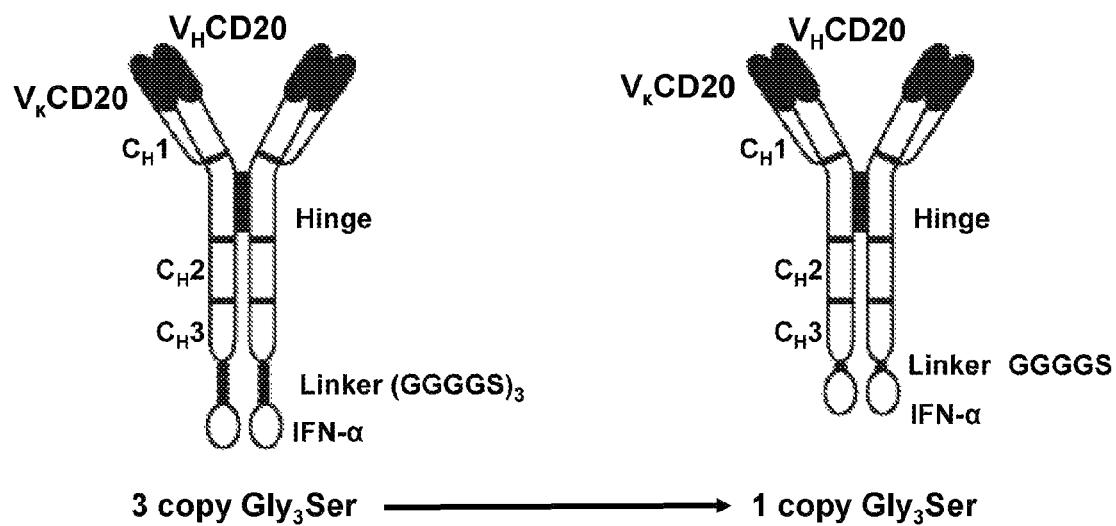
*Fig.6C*



*Fig.7*



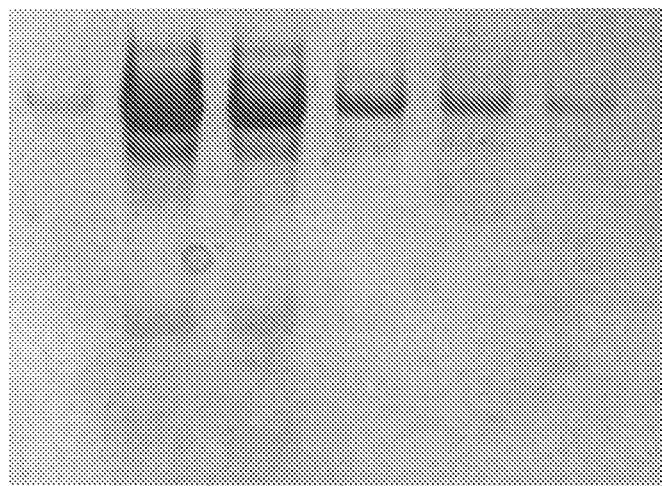
*Fig. 8*



*Fig. 9*

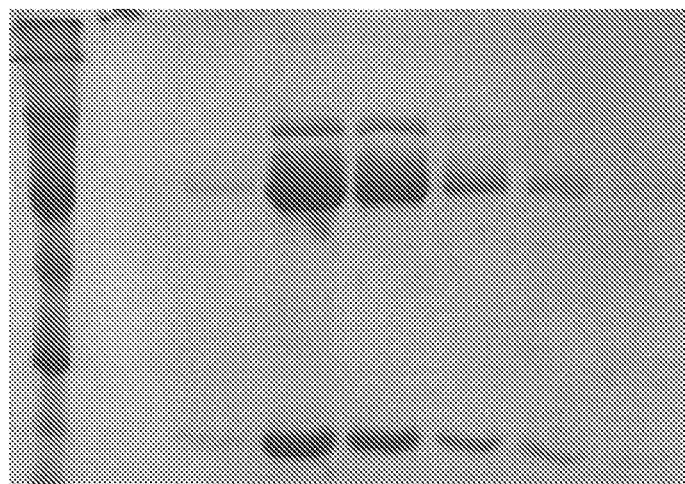
**A**

lane # 1 2 3 4 5 6



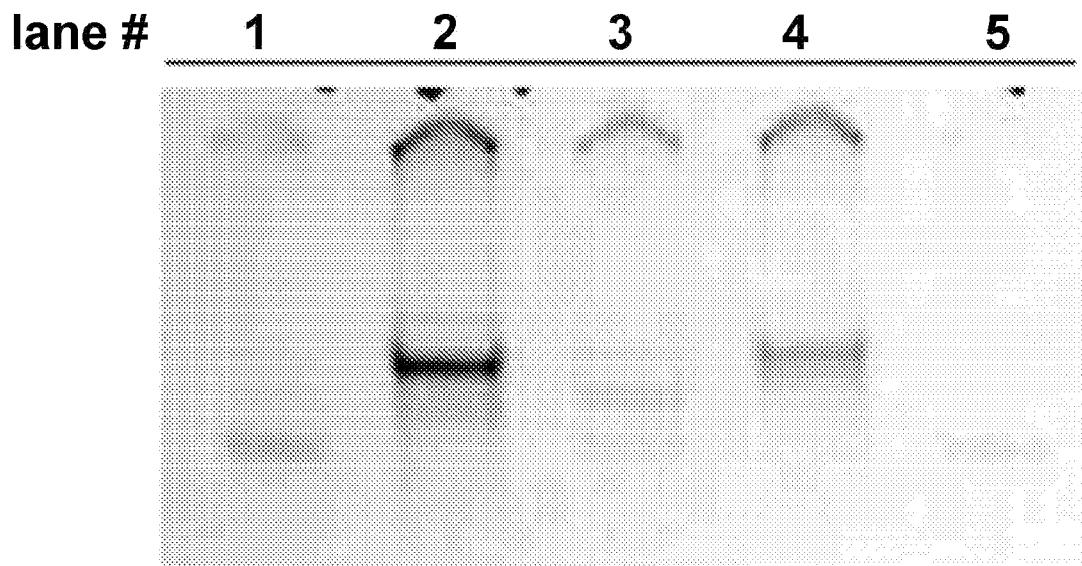
phosphate gel (non-reducing)

MW B  
marker 1 2 3 4 5 6 7

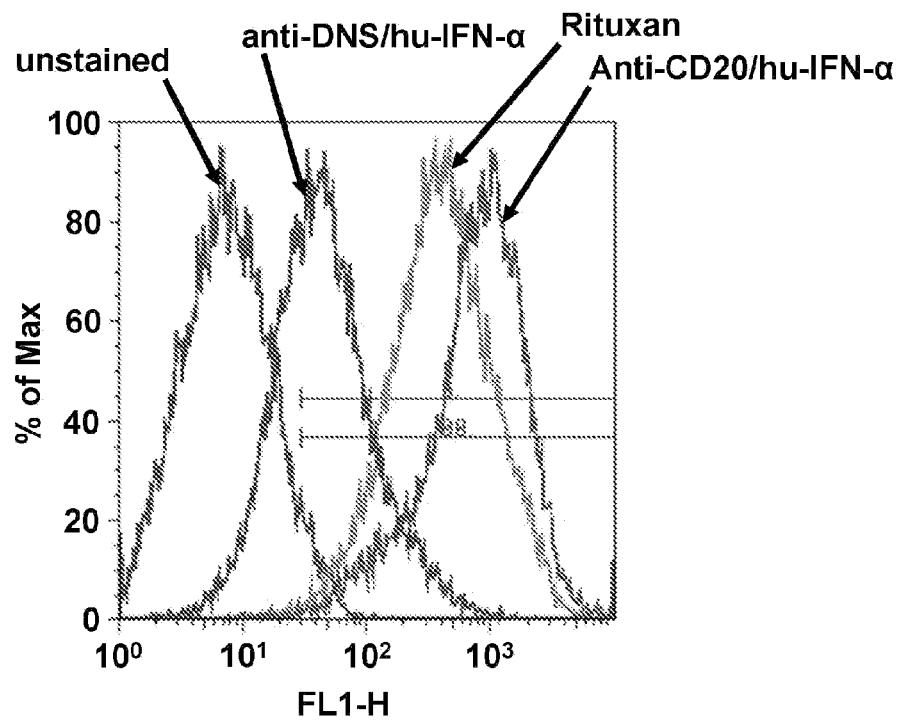


tris-glycine gel (reducing)

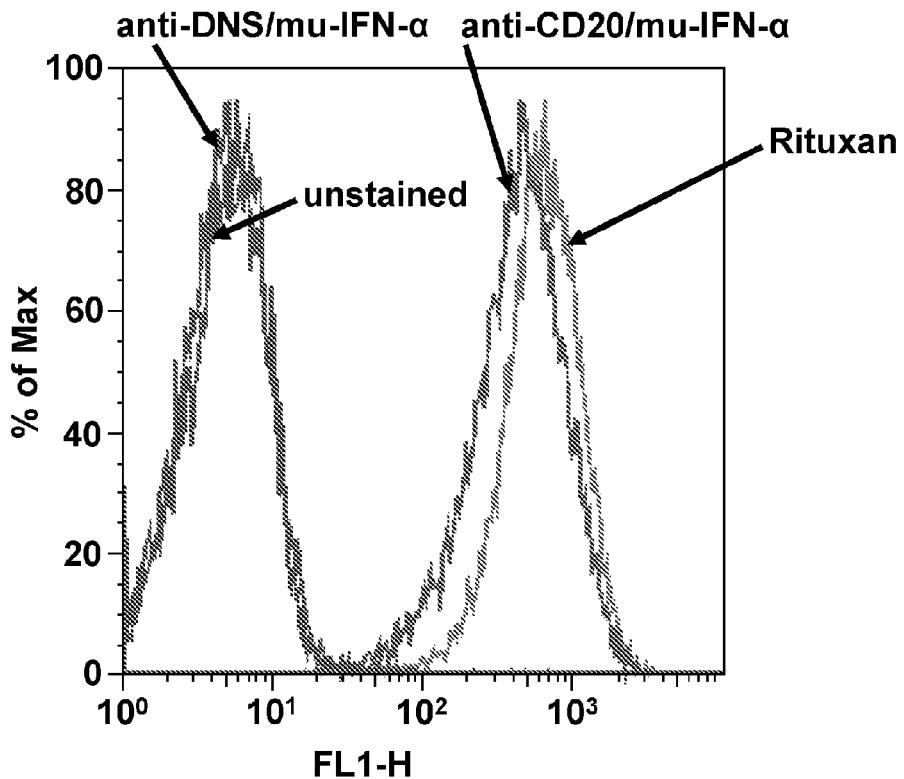
***Fig. 10***



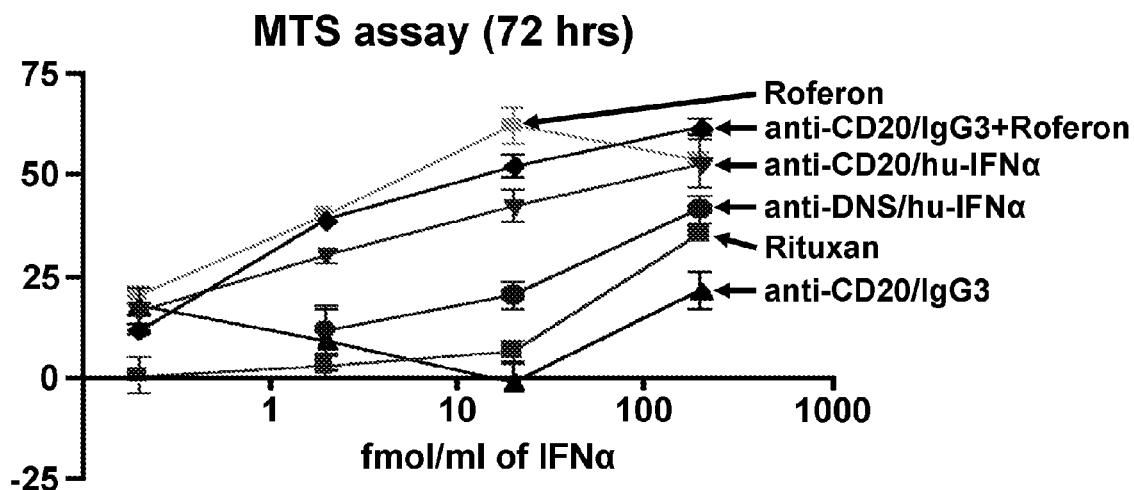
*Fig. 11*



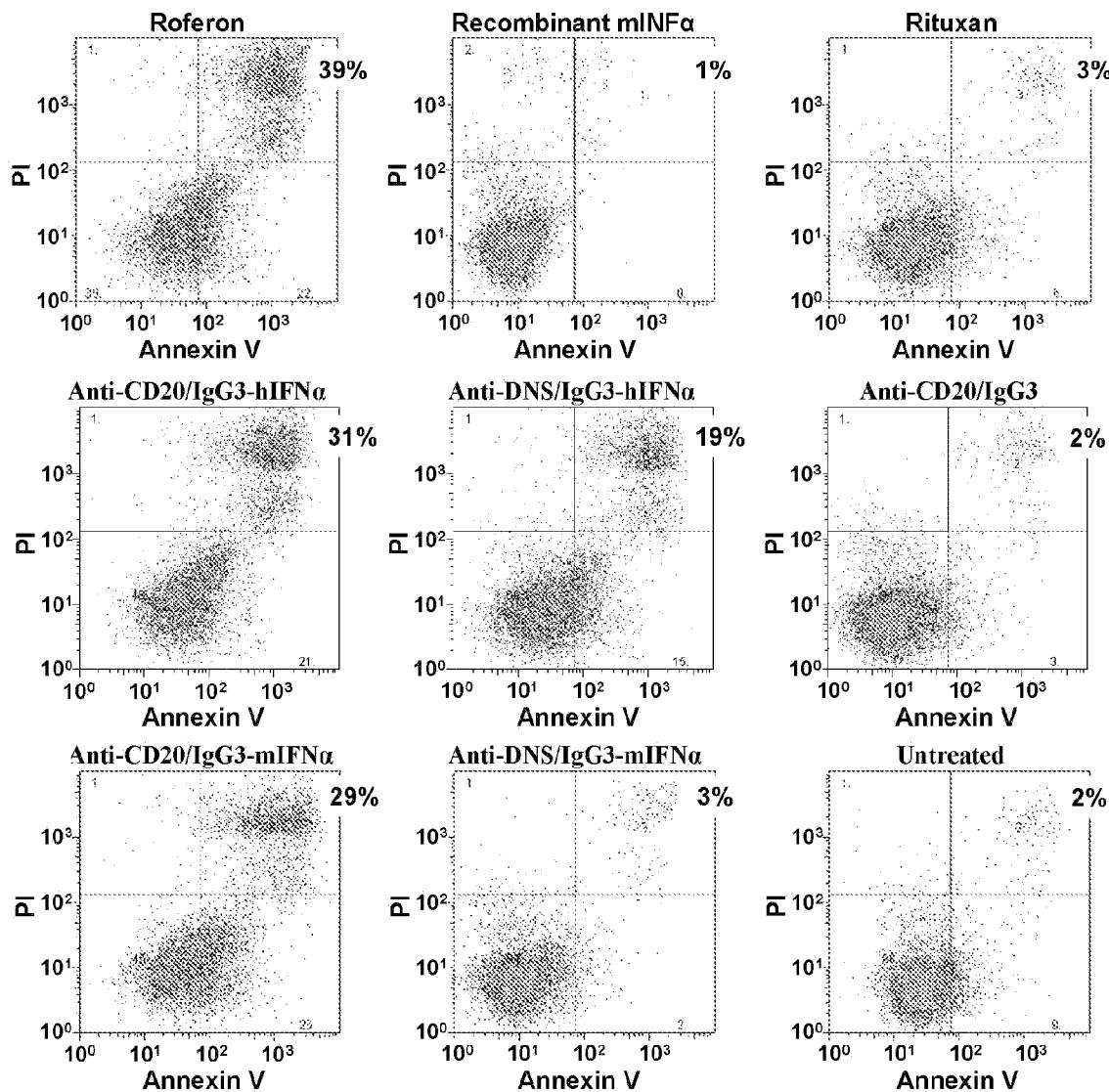
*Fig. 12*



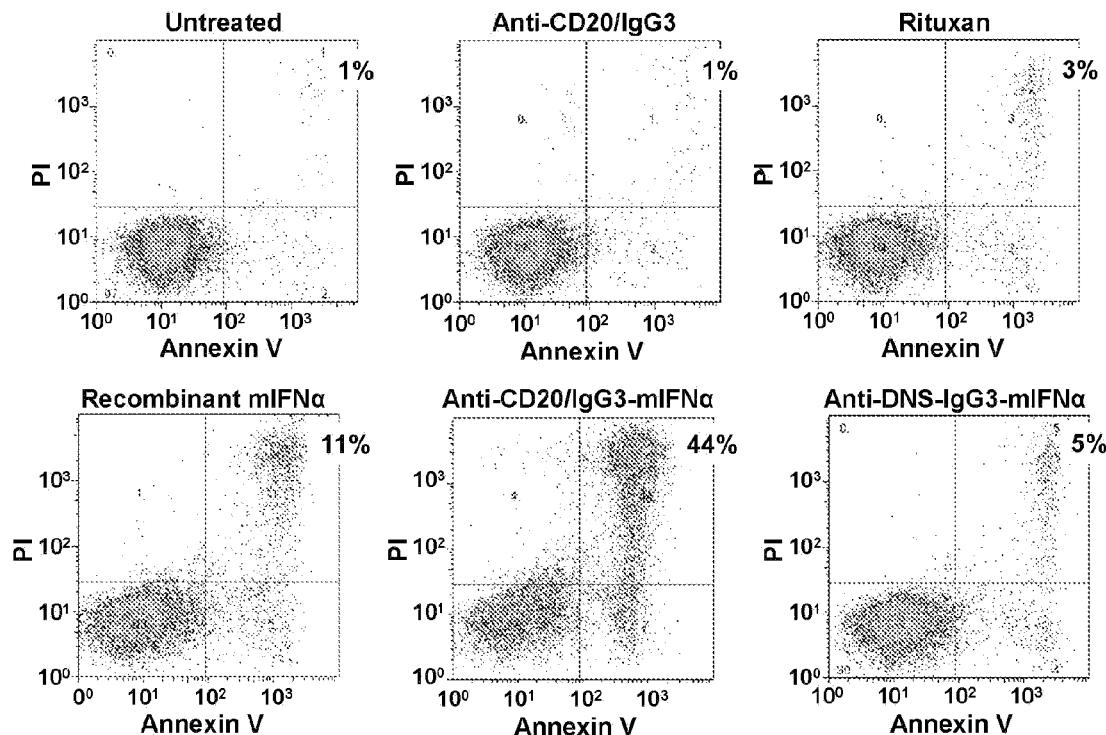
*Fig. 13*



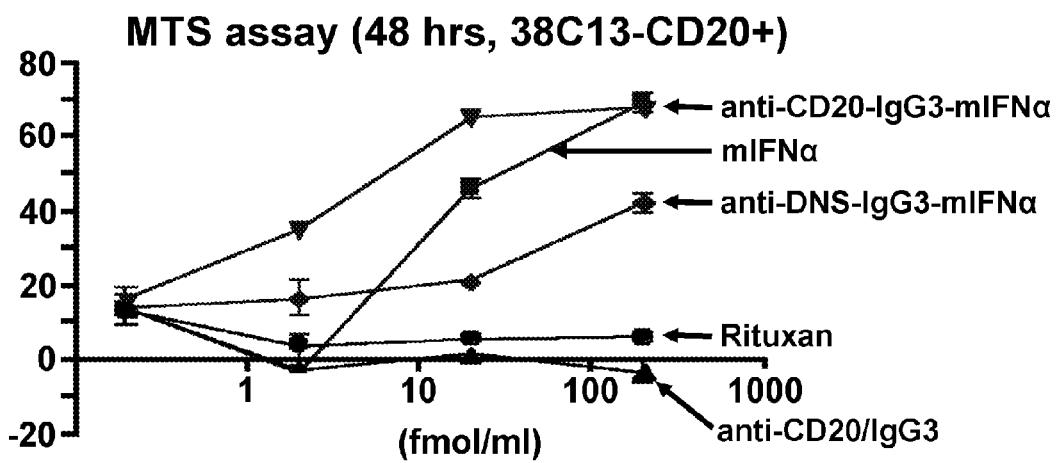
*Fig. 14*



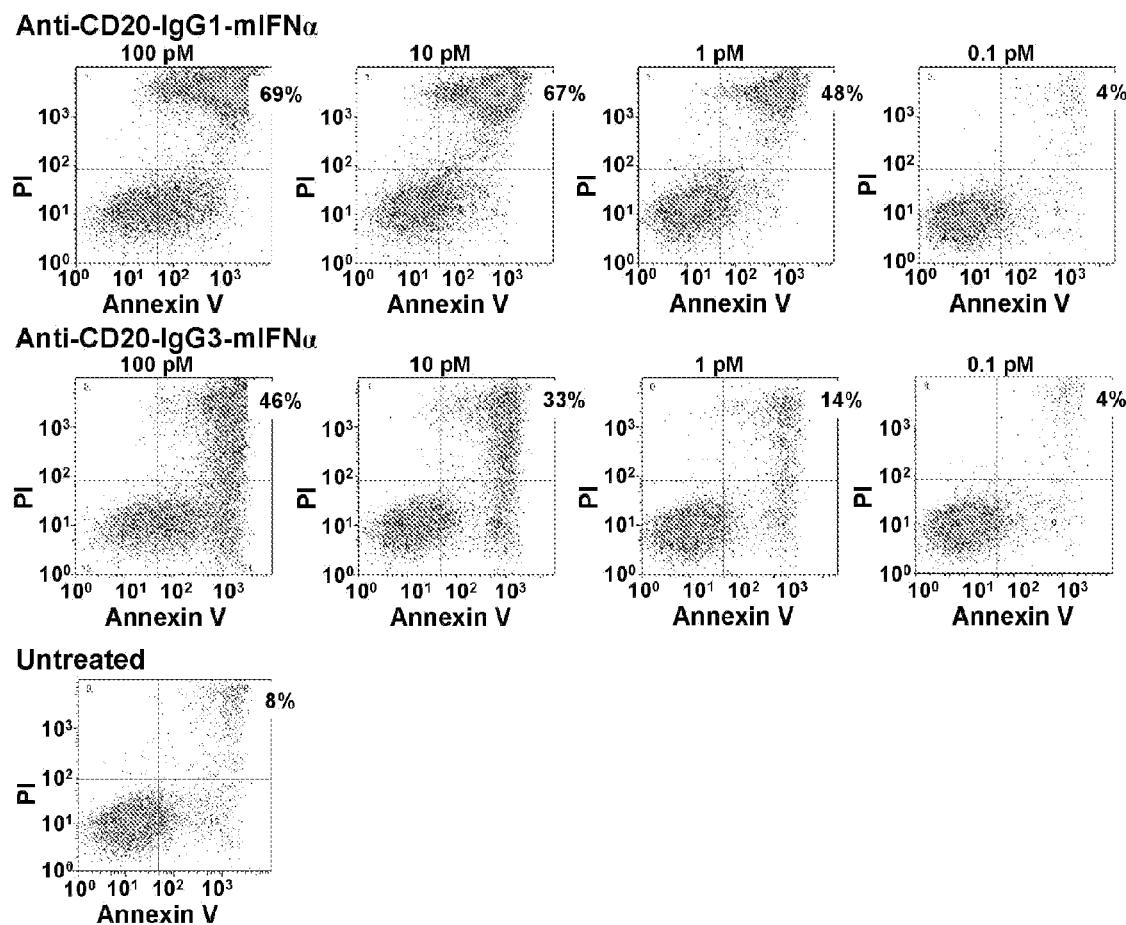
*Fig. 15*



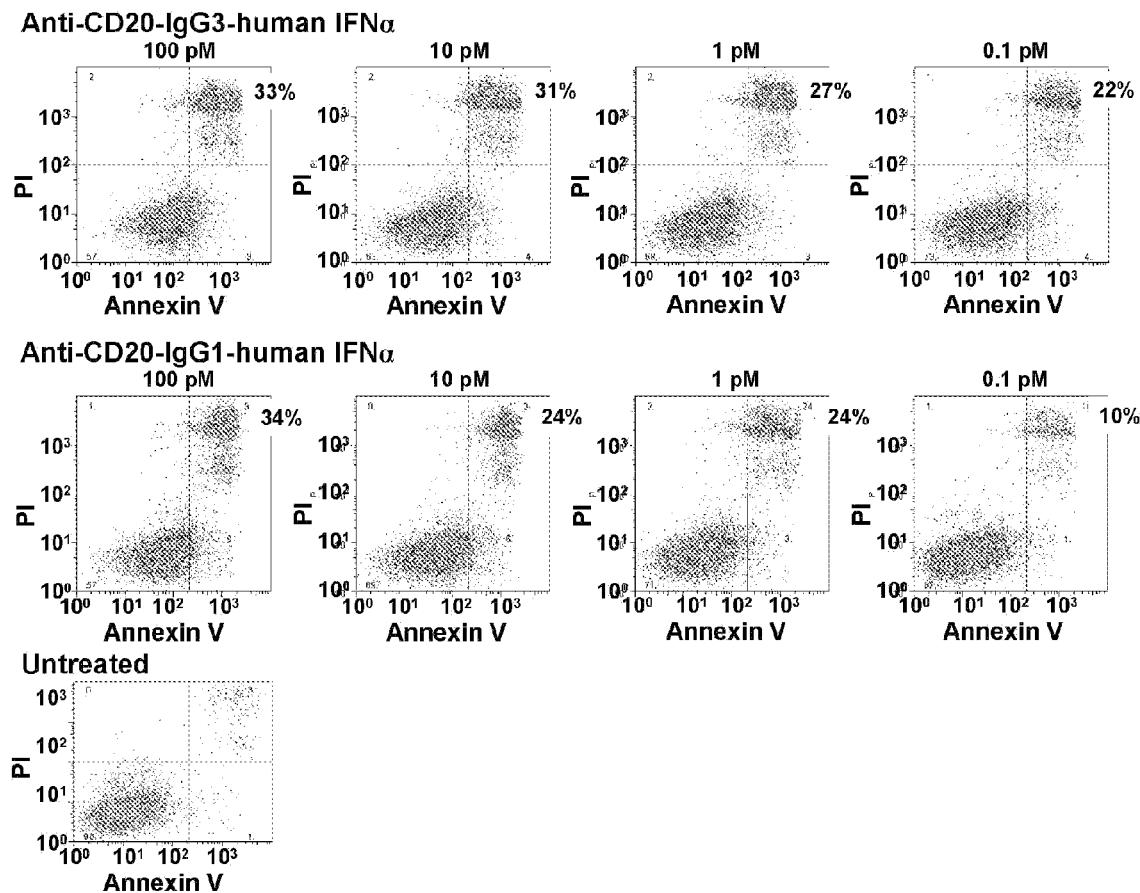
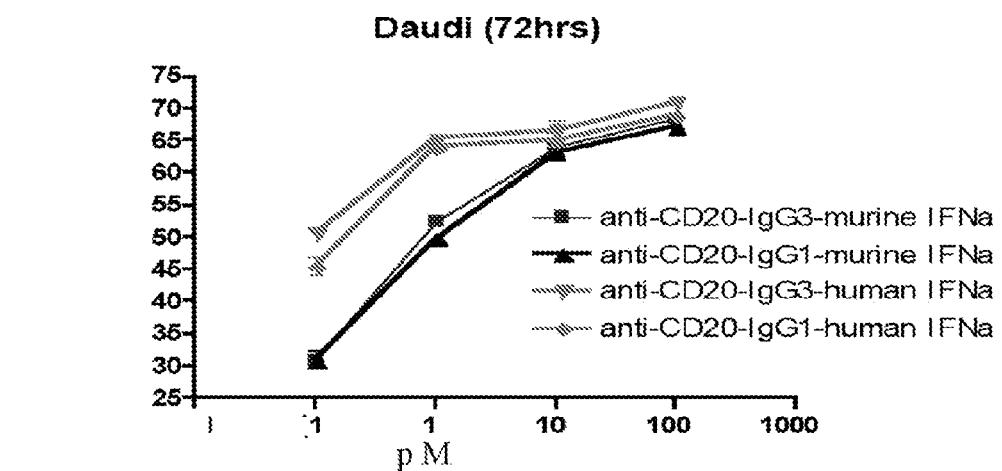
**Fig. 16**

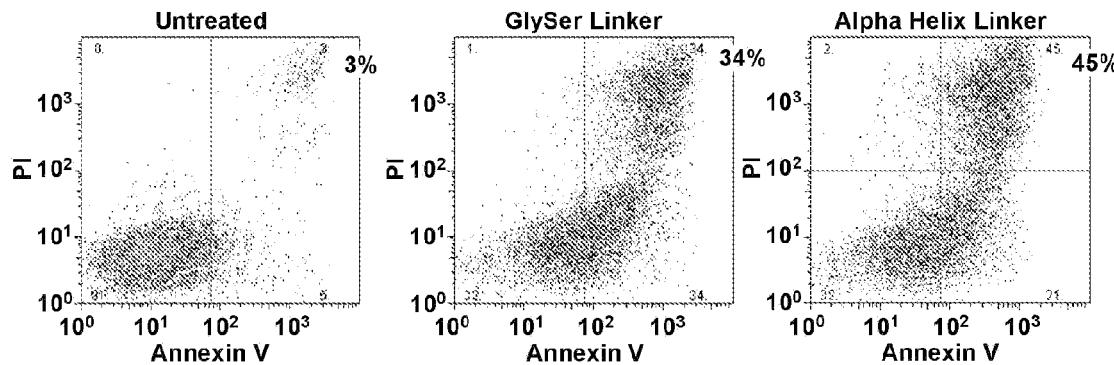
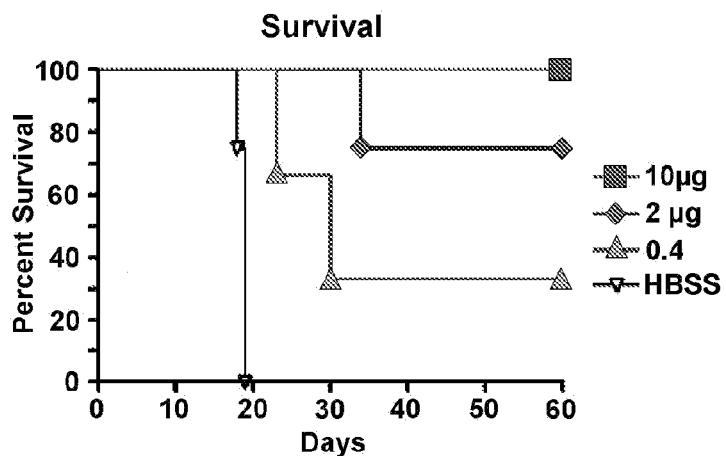


**Fig. 17**



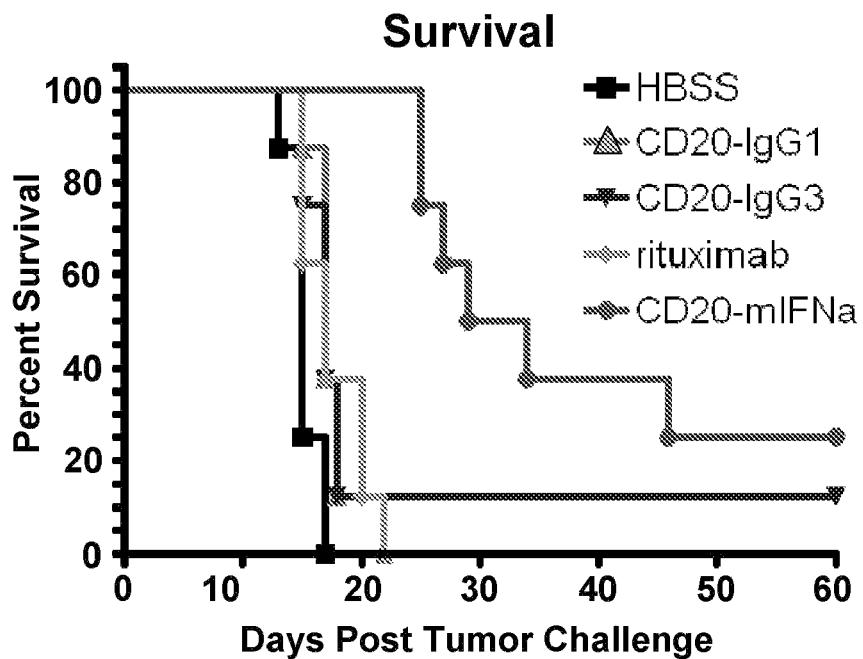
***Fig. 18***

**Fig. 19****Fig. 20**

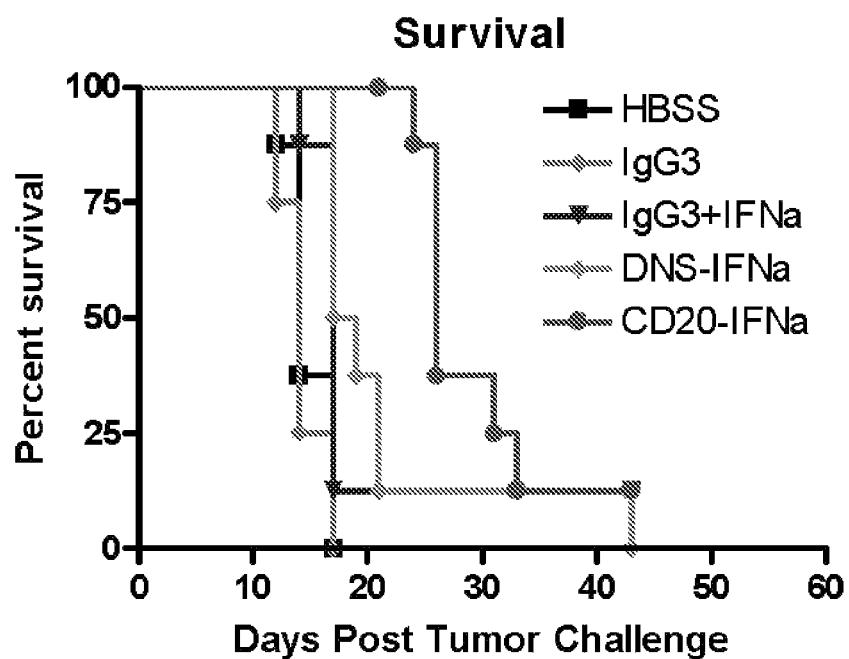
**Fig. 21**

Comparison of Survival curves	HBSS vs 10 ug	Comparison of Survival curves	HBSS vs 2 ug
Logrank Test		Logrank Test	
Chi square	6.628	Chi square	6.628
df	1	df	1
P value	0.0100	P value	0.0100
P value summary	*	P value summary	*
Are the survival curves different?	yes	Are the survival curves different?	yes
Comparison of Survival curves	HBSS vs 0.4 ug	Comparison of Survival curves	0.4 ug vs 2 ug
Logrank Test		Logrank Test	
Chi square	6.352	Chi square	3.282
df	1	df	1
P value	0.0207	P value	0.0701
P value summary	*	P value summary	ns
Are the survival curves different?	yes	Are the survival curves different?	no

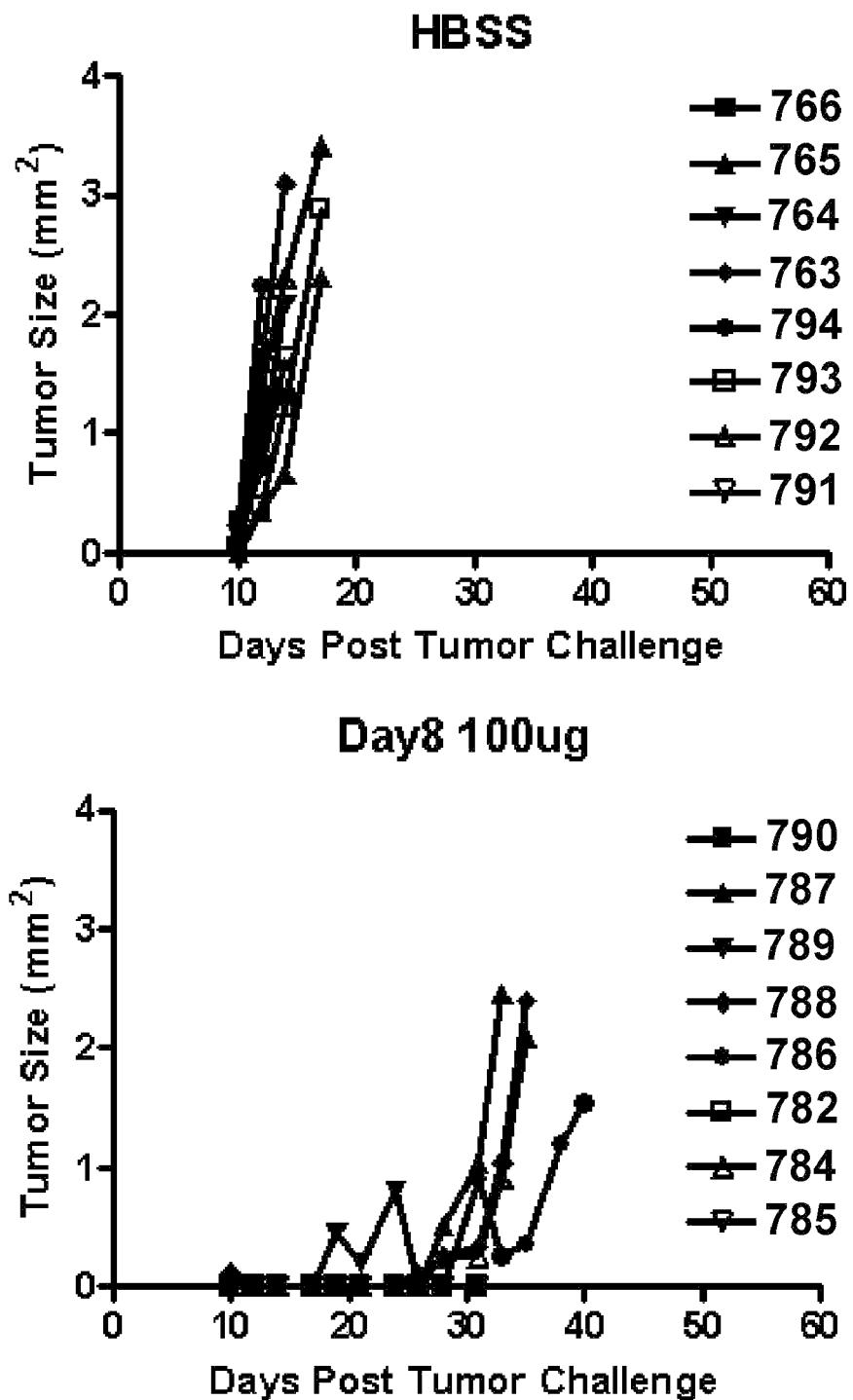
**Fig. 22**



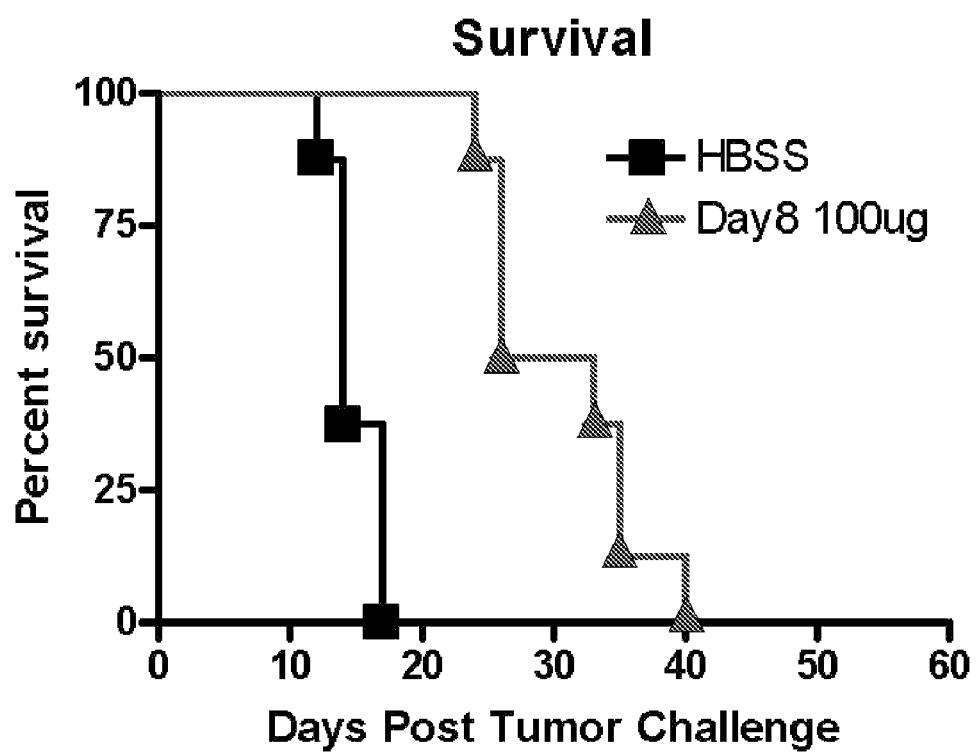
*Fig. 23*



*Fig. 24*



***Fig. 25***



*Fig. 26*

**1**

**INTERFERON-ANTIBODY FUSION  
PROTEINS DEMONSTRATING POTENT  
APOPTOTIC AND ANTI-TUMOR ACTIVITIES**

**CROSS-REFERENCE TO RELATED  
APPLICATIONS**

This application is a 371 National Phase of PCT/US2008/077074, filed on Sep. 19, 2008, which claims priority to and benefit of U.S. Ser. No. 60/994,717, filed on Sep. 21, 2007, both of which are incorporated herein by reference in their entirety for all purposes.

**STATEMENT OF GOVERNMENTAL SUPPORT**

This invention was made with Government support under Grant No. CA087990, awarded by the National Institutes of Health. The Government has rights in the invention.

**FIELD OF THE INVENTION**

This invention pertains to the field of oncology. Chimeric constructs are provided that have significant anti-cancer activity.

**BACKGROUND OF THE INVENTION**

Although spontaneous immune responses against tumor-associated antigens (TAAs) (Hrouda et al. (1999) *Semin. Oncol.* 26: 455-471) can be detected (Disis et al. (1997) *J. Clin. Oncol.* 15: 3363-3367), malignant cells causing disease fail to elicit an immune response that leads to rejection. Many studies have demonstrated that it is possible to enhance the immunogenicity of tumor cells by introducing immunostimulatory molecules such as cytokines and costimulatory molecules into them (Dranoff and Mulligan (1995) *Adv. Immunol.* 58: 417-454; Hrouda et al. (1999) *Semin. Oncol.* 26: 455-471; Hurford et al. (1995) *Nat. Genet.* 10: 430-435); however, effective gene transfer still remains a challenge. In addition, eradication of residual cancer cells may require the targeting of widely scattered micrometastatic tumor deposits that are not accessible to direct gene transfer.

Both the innate and the adaptive immune responses are essential for providing protection against infectious pathogens and tumors. The cross-talk between innate and adaptive immunity is regulated by interactions between cells and cytokines. Cytokines produced by cells of the innate immune system can, directly or indirectly, activate the cells of the adaptive immune response and can play an important role in eliciting protective antitumor immunity (Belardelli and Ferrantini (2002) *Trends Immunol.* 23: 201-208). Central to the activation of the innate immune system is the detection of bacterial products or “danger” signals that lead to the release of proinflammatory cytokines, such as IFN- $\alpha$ , TNF- $\alpha$ , and IL-1.

IFN- $\alpha$  is a proinflammatory cytokine with potent antiviral and immunomodulatory activities and is a stimulator of differentiation and activity of dendritic cells (DCs) (Santini et al. (2000) *J. Exp. Med.* 191: 1777-1788). Type I IFNs (IFN- $\alpha$  and IFN- $\beta$ ) have multiple effects on the immune response (Theofilopoulos et al. (2005) *Annu. Rev. Immunol.* 23: 307-336). IFN- $\alpha$  plays a role in the differentiation of Th1 cells (Finkelman et al. (1991) *J. Exp. Med.* 174: 1179-1188) and the long-term survival of CD8+ T cells in response to specific antigens (Tough et al. (1996) *Science* 272: 1947-1950).

Multiple studies have shown that IFNs are also capable of exerting antitumor effects in both animal models (Ferrantini

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et al. (1994) *J. Immunol.* 153: 4604-4615) and cancer patients (14. Guterman et al. (1980) *Ann. Intern. Med.* 93: 399-406). In addition to enhancing the adaptive antitumor immune response, IFN- $\alpha$  can increase expression of the tumor suppressor gene P53 (Takaoka et al. (2003) *Nature* 424: 516-523), inhibit angiogenesis (Sidky and Borden (1987) *Cancer Res.* 47: 5155-5161), and prime apoptosis (Rodriguez-Villanueva and McDonnell (1995) *Int. J. Cancer* 61: 110-11417) in tumor cells. Although these properties suggest that IFN- $\alpha$  should be an effective therapeutic for the treatment of cancer, its short half-life and systemic toxicity have limited its usage.

**SUMMARY OF THE INVENTION**

15 In various embodiments this invention pertains to the discovery that attaching an interferon to a targeting moiety (e.g., a molecule that specifically and/or preferentially binds a marker on or associated with a cell) substantially improves the therapeutic efficacy of the interferon and appears to 20 reduce systemic toxicity. Accordingly, in various embodiments, this invention provides constructs comprising an interferon attached to a targeting moiety and uses of such constructs to specifically and/or preferentially inhibit the growth or proliferation or even to kill certain target cells (e.g., cancer cells).

Accordingly, in certain embodiments, a chimeric construct is provided where the construct comprises an interferon (e.g., interferon-alpha, interferon-beta, interferon-gamma, etc.) attached to a targeting moiety that binds to a tumor associated antigen (TAA), where the construct when contacted to a tumor cell results in the killing or inhibition of growth or proliferation of the tumor cell. In certain embodiments a chimeric construct is provided where the construct comprises an interferon attached to a targeting moiety that binds to a cell surface marker or a cell-associated marker, where the targeting is not attached to the interferon by a (Gly<sub>4</sub>Ser)<sub>3</sub> (SEQ ID NO:31) linker. In various embodiments the interferon is a type 1 interferon. In various embodiments the interferon is a type 2 interferon. In various embodiments the is an interferon alpha, an interferon-beta, or an interferon-gamma. In certain embodiments the targeting moiety is an antibody that binds a tumor associated antigen. In certain embodiments the targeting moiety is chemically coupled to the interferon. In certain embodiments the targeting moiety is joined to the interferon with a peptide linker. In certain embodiments the peptide linker is fewer than 15, fewer than 14, fewer than 12, fewer than 11, fewer than 10, fewer than 9, fewer than 8, fewer than 7, fewer than 6, fewer than 5, fewer than 4, fewer than 3, or fewer than 2 amino acids in length. In certain embodiments the linker is 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid in length. In certain embodiments the linker is not (Gly<sub>4</sub>Ser)<sub>3</sub> (SEQ ID NO:31). In certain embodiments the linker is a linker that is resistant or substantially resistant to proteolysis. In certain embodiments the peptide linker is 55 Gly<sub>4</sub>Ser (SEQ ID NO:32). In certain embodiments the linker comprises or consists of an amino acid sequence found in Table 2. In certain embodiments the construct is a recombinantly expressed fusion protein. In certain embodiments the antibody specifically binds a marker selected from the group 60 consisting of EGFR, HER4, HER3, HER2/neu, MUC-1, G250, mesothelin, gp100, tyrosinase, and MAGE. In certain embodiments the targeting moiety is an antibody that binds CD20. In certain embodiments the targeting moiety is a single chain antibody that comprises the CDRs and/or the variable 65 regions from an antibody selected from the group consisting of anti-CD20 (Rituximab), Ibritumomab tiuxetan, tositumomab, AME-133v, Ocrelizumab, Ofatumumab, TRU-015,

IMMU-106, and the like. In various embodiments the targeting moiety is an antibody that binds HER2. In certain embodiments the antibody is a C6 antibody. In certain embodiments the antibody comprises the VH and VL CDRs or VH and VL domains of C6MH3-B1. In various embodiments the antibody is an IgG (e.g., IgG1, IgG3, etc.), an IgE, a single chain Fv (scFv), a FAB, a (Fab')<sub>2</sub>, an (ScFv)<sub>2</sub>, and the like. In certain embodiments the antibody is an antibody selected from the group consisting of Rituxan, IF5, B1, 1H4, CD19, B4, B43, FVS191, hLL2, LL2, RFB4, M195, HuM195, AT13/5, HERCEPTIN®, 4D5, HuCC49, HUCC39ΔCH2 B72.3, 12C10, IG5, H23, BM-2, BM-7, 12H12, MAM-6, and HMFG-1. In certain embodiments the antibody is an antibody that binds a member of the EGF receptor family. In certain embodiments the antibody is selected from the group consisting of C6.5, C6ML3-9, C6MH3-B1, C6-B1D2, F5, HER3.A5, HER3.F4, HER3.H1, HER3.H3, HER3.E12, HER3.B12, EGFR.E12, EGFR.C10, EGFR.B11, EGFR.E8, HER4.B4, HER4.G4, HER4.F4, HER4.A8, HER4.B6, HER4.D4, HER4.D7, HER4.D11, HER4.D12, HER4.E3, HER4.E7, HER4.F8 and HER4.C7. In certain embodiments the construct comprises an anti-HER2 IgG1 antibody attached to an interferon.

Also provided are pharmaceutical formulations. In various embodiments the formulations comprise a chimeric construct comprising an interferon attached to a targeting moiety. In certain embodiments the chimeric construct comprises a construct as described above (and/or herein below) (e.g., an anti-CD20-Interferon, and anti-HER2-interferon, etc.). In certain embodiments the formulation is a unit dosage formulation. In certain embodiments the formulation is a formulation for parenteral administration. In certain embodiments the formulation is a formulated for administration via a route selected from the group consisting of oral administration, intravenous administration, intramuscular administration, direct tumor administration, inhalation, rectal administration, vaginal administration, transdermal administration, and subcutaneous depot administration.

In various embodiments methods are provided for inhibiting growth and/or proliferation of a cancer cell. The methods typically involve contacting the cancer cell with a chimeric construct as described herein. In certain embodiments the cancer cell is a metastatic cell, and/or a cell is in a solid tumor. In certain embodiments the cancer cell is a breast cancer cell. In certain embodiments the cancer cell is a B cell lymphoma. In certain embodiments the cancer cell is a cell produced by a cancer selected from the group consisting of a B cell lymphoma, lung cancer, a bronchus cancer, a colorectal cancer, a prostate cancer, a breast cancer, a pancreas cancer, a stomach cancer, an ovarian cancer, a urinary bladder cancer, a brain or central nervous system cancer, a peripheral nervous system cancer, an esophageal cancer, a cervical cancer, a melanoma, a uterine or endometrial cancer, a cancer of the oral cavity or pharynx, a liver cancer, a kidney cancer, a biliary tract cancer, a small bowel or appendix cancer, a salivary gland cancer, a thyroid gland cancer, an adrenal gland cancer, an osteosarcoma, a chondrosarcoma, a liposarcoma, a testes cancer, and a malignant fibrous histiocytoma. In various embodiments the contacting comprises systemically administering the chimeric moiety to a mammal. In certain embodiments the contacting comprises administering the chimeric moiety directly into a tumor site. In certain embodiments the contacting comprises intravenous administration of the chimeric moiety. In certain embodiments the cancer cell is a cancer cell in a human or in a non-human mammal.

In certain embodiments nucleic acids are provided that encode the chimeric constructs described herein. In various embodiments the nucleic acid encodes a fusion protein com-

prising an interferon attached to an anti-EGFR family member antibody, an anti-HER2 antibody, an anti-C6 single-chain antibody, or to an anti-CD20 single chain antibody. In various embodiments the interferon encoded by the nucleic acid is a type I interferon. In certain embodiments the interferon is IFN- $\alpha$  or interferon- $\beta$ . In various embodiments the nucleic encodes an antibody that comprises the VH and VL CDRs of C6MH3-B1. In various embodiments nucleic acid encodes a peptide linker (e.g., as described herein) attaching the antibody to the interferon. In certain embodiments the nucleic acid encodes the CDRs and/or the variable regions for anti-CD20 (Rituximab).

Also provided is a cell comprising a nucleic acid described above, that encodes a chimeric construct. In certain embodiments the cell expresses the chimeric construct.

In various embodiments this invention provides the use of a chimeric construct as described herein in the manufacture of a medicament to inhibit the growth and/or proliferation of a cancer cell.

In certain embodiments, the methods and constructs of this invention specifically exclude constructs using any of the antibodies disclosed in U.S. Patent Publication No: US 2002/0193569 A1. In certain embodiments the methods and constructs of this invention specifically exclude constructs incorporating an anti-CD20 antibody. In certain embodiments the methods and constructs of this invention specifically exclude constructs incorporating antibodies that bind to any of the following targets: CD19, CD20, CD22, CD33, CD38, EGFR, HM1.24, phosphatidyl serine antigen, HER-2, TAG-72, and/or MUC-1. In certain embodiments the constructs described herein can be used in the treatment of pathologies such as multiple sclerosis, HCV mediated vasculitis, and the like.

#### DEFINITIONS

The terms "polypeptide", "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers. The term also includes variants on the traditional peptide linkage joining the amino acids making up the polypeptide. Preferred peptides", "polypeptides", and "proteins" are chains of amino acids whose alpha carbons are linked through peptide bonds. The terminal amino acid at one end of the chain (amino terminal) therefore has a free amino group, while the terminal amino acid at the other end of the chain (carboxy terminal) has a free carboxyl group. As used herein, the term "amino terminus" (abbreviated N-terminus) refers to the free  $\alpha$ -amino group on an amino acid at the amino terminal of a peptide or to the  $\alpha$ -amino group (imino group when participating in a peptide bond) of an amino acid at any other location within the peptide. Similarly, the term "carboxy terminus" refers to the free carboxyl group on the carboxy terminus of a peptide or the carboxyl group of an amino acid at any other location within the peptide. Peptides also include essentially any polyamino acid including, but not limited to peptide mimetics such as amino acids joined by an ether as opposed to an amide bond.

As used herein, an "antibody" refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes or fragments of immunoglobulin genes. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as myriad immunoglobulin variable region

genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively.

A typical immunoglobulin (antibody) structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain ( $V_L$ ) and variable heavy chain ( $V_H$ ) refer to these regions of the light and heavy chains respectively.

Antibodies exist as intact immunoglobulins or as a number of well characterized fragments produced by digestion with various peptidases or expressed de novo. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce  $F(ab')_2$ , a dimer of Fab which itself is a light chain joined to  $V_H C_H 1$  by a disulfide bond. The  $F(ab')_2$  may be reduced under mild conditions to break the disulfide linkage in the hinge region thereby converting the  $(Fab')_2$  dimer into an Fab' monomer. The Fab' monomer is essentially an Fab with part of the hinge region (see, *Fundamental Immunology*, W. E. Paul, ed., Raven Press, N.Y. (1993), for a more detailed description of other antibody fragments). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such Fab' fragments may be synthesized de novo either chemically or by utilizing recombinant DNA methodology. Thus, the term antibody, as used herein also includes antibody fragments either produced by the modification of whole antibodies or synthesized de novo using recombinant DNA methodologies, including, but are not limited to,  $Fab'_2$ , IgG, IgM, IgA, IgE, scFv, dAb, nanobodies, unibodies, and diabodies. In various embodiments preferred antibodies include, but are not limited to  $Fab'_2$ , IgG, IgM, IgA, IgE, and single chain antibodies, more preferably single chain Fv (scFv) antibodies in which a variable heavy and a variable light chain are joined together (directly or through a peptide linker) to form a continuous polypeptide.

In certain embodiments antibodies and fragments used in the constructs of the present invention can be bispecific. Bispecific antibodies or fragments can be of several configurations. For example, bispecific antibodies may resemble single antibodies (or antibody fragments) but have two different antigen binding sites (variable regions). In various embodiments bispecific antibodies can be produced by chemical techniques (Kranz et al. (1981) *Proc. Natl. Acad. Sci., USA*, 78: 5807), by "polydoma" techniques (see, e.g., U.S. Pat. No. 4,474,893), or by recombinant DNA techniques. In certain embodiments bispecific antibodies of the present invention can have binding specificities for at least two different epitopes at least one of which is a tumor associate antigen. In various embodiments the antibodies and fragments can also be heteroantibodies. Heteroantibodies are two or more antibodies, or antibody binding fragments (e.g., Fab) linked together, each antibody or fragment having a different specificity.

An "antigen-binding site" or "binding portion" refers to the part of an immunoglobulin molecule that participates in antigen binding. The antigen binding site is formed by amino acid residues of the N-terminal variable ("V") regions of the heavy ("H") and light ("L") chains. Three highly divergent stretches within the V regions of the heavy and light chains are referred to as "hypervariable regions" which are interposed between more conserved flanking stretches known as "framework regions" or "FRs". Thus, the term "FR" refers to amino acid

sequences that are naturally found between and adjacent to hypervariable regions in immunoglobulins. In an antibody molecule, the three hypervariable regions of a light chain and the three hypervariable regions of a heavy chain are disposed relative to each other in three dimensional space to form an antigen binding "surface". This surface mediates recognition and binding of the target antigen. The three hypervariable regions of each of the heavy and light chains are referred to as "complementarity determining regions" or "CDRs" and are characterized, for example by Kabat et al. *Sequences of proteins of immunological interest*, 4th ed. U.S. Dept. Health and Human Services, Public Health Services, Bethesda, Md. (1987).

The term "interferon" refers to a full-length interferon or to an interferon fragment (truncated interferon) or interferon mutant, that substantially retains the biological activity of the full length wild-type interferon (e.g., retains at least 80%, preferably at least 90%, more preferably at least 95%, 98%, or 99% of the full-length antibody). Interferons include type I interferons (e.g., interferon-alpha and interferon-beta) as well as type II interferons (e.g., interferon-gamma). The interferon (e.g., IFN- $\alpha$ ) can be from essentially any mammalian species. In certain preferred embodiments, the interferon is from a species selected from the group consisting of human, equine, bovine, rodent, porcine, lagomorph, feline, canine, murine, caprine, ovine, a non-human primate, and the like. In various embodiments the mutated interferon comprises one or more amino acid substitutions, insertions, and/or deletions.

An anti-HER2/neu antibody is an antibody that specifically or preferentially binds a HER2/neu receptor.

As used herein, the term "subject" refers to a human or non-human animal, including, but not limited to, a cat, dog, horse, pig, cow, sheep, goat, rabbit, mouse, rat, or monkey.

The term "C6 antibody", as used herein refers to antibodies derived from C6.5 whose sequence is expressly provided, for example, in U.S. Pat. Nos. 6,512,097 and 5,977,322, and in PCT Publication WO 97/00271. C6 antibodies preferably have a binding affinity of about  $1.6 \times 10^{-8}$  or better for HER2/neu. In certain embodiments C6 antibodies are derived by screening (for affinity to c-erbB-2/HER2/neu) a phage display library in which a known C6 variable heavy ( $V_H$ ) chain is expressed in combination with a multiplicity of variable light ( $V_L$ ) chains or conversely a known C6 variable light chain is expressed in combination with a multiplicity of variable heavy ( $V_H$ ) chains. C6 antibodies also include those antibodies produced by the introduction of mutations into the variable heavy or variable light complementarity determining regions (CDR1, CDR2 or CDR3), e.g., as described in U.S. Pat. Nos. 6,512,097 and 5,977,322, and in PCT Publication WO 97/00271. In addition, C6 antibodies include those antibodies produced by any combination of these modification methods as applied to C6.5 and its derivatives.

An "anti-EGFR family antibody" refers to an antibody that specifically binds to a member of the epidermal growth factor receptor family (e.g., an antibody that binds to ErbB-1, also named epidermal growth factor receptor (EGFR), ErbB-2, also named HER2 in humans and neu in rodents, ErbB-3, also named HER3, and/or to ErbB-4, also named HER4). Illustrative anti-EGFR family antibodies include, but are not limited to antibodies such as C6.5, C6ML3-9, C6MH3-B1, C6-B1D2, F5, HER3.A5, HER3.F4, HER3.H1, HER3.H3, HER3.E12, HER3.B12, EGFR.E12, EGFR.C10, EGFR.B11, EGFR.E8, HER4.B4, HER4.G4, HER4.F4, HER4.A8, HER4.B6, HER4.D4, HER4.D7, HER4.D11, HER4.D12, HER4.E3, HER4.E7, HER4.F8 and HER4.C7 and the like

(see, e.g., U.S. Patent publications US 2006/0099205 A1 and US 2004/0071696 A1 which are incorporated herein by reference).

A single chain Fv ("sFv" or "scFv") polypeptide is a covalently linked  $V_H$ : $V_L$  heterodimer which, in certain embodiments, may be expressed from a nucleic acid including  $V_H$ - and  $V_L$ -encoding sequences either joined directly or joined by a peptide-encoding linker. Huston, et al. *Proc. Nat. Acad. Sci. USA*, 85: 5879-5883 (1988). A number of structures for converting the naturally aggregated, but chemically separated light and heavy polypeptide chains from an antibody V region into an sFv molecule that will fold into a three dimensional structure substantially similar to the structure of an antigen-binding site. See, e.g. U.S. Pat. Nos. 5,091,513 and 5,132,405, and 4,956,778.

"CD20" is a non-glycosylated phosphoprotein expressed on the surface of mature B-cells (see, e.g., Cragg et al. (2005) *Curr. Dir. Autoimmun.*, 8: 140-174). It is also found on B-cell lymphomas, hairy cell leukemia, B-cell chronic lymphocytic leukemia, on skin/melanoma cancer stem cells, and the like.

The phrase "inhibition of growth and/or proliferation" of a cancer cell refers to decrease in the growth rate and/or proliferation rate of a cancer cell. In certain embodiments this includes death of a cancer cell (e.g. via apoptosis). In certain embodiments this term also refers to inhibiting the growth and/or proliferation of a solid tumor and/or inducing tumor size reduction or elimination of the tumor.

The term "cancer marker" refers to biomolecules such as proteins, carbohydrates, glycoproteins, and the like that are exclusively or preferentially or differentially expressed on a cancer cell and/or are found in association with a cancer cell and thereby provide targets preferential or specific to the cancer. In various embodiments the preferential expression can be preferential expression as compared to any other cell in the organism, or preferential expression within a particular area of the organism (e.g. within a particular organ or tissue).

#### BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-1o show the nucleic acid and amino acid sequences for various constructs described herein. FIG. 1A shows amino acid sequences for anti-HER2/neu IgG3 heavy chain-IFN- $\alpha$  (SEQ ID NO:1) and anti-HER2/neu IgG3 light chain (SEQ ID NO:2). Single underline is linker, double underline is murine IFN- $\alpha$ , no underline is anti-HER2/neu. FIG. 1B:  $\alpha$ CD20 light chain, nucleic acid (SEQ ID NO:3), amino acid sequence (SEQ ID NO:4); FIG. 1C:  $\alpha$ CD20-IgG3-muIFN $\alpha$  Gly<sub>4</sub>Ser linker, nucleic acid (SEQ ID NO:5), amino acid sequence (SEQ ID NO:6); FIG. 1D:  $\alpha$ CD20-IgG3-muIFN $\alpha$  alpha helical linker, nucleic acid (SEQ ID NO:7), amino acid sequence (SEQ ID NO:8); FIG. 1E:  $\alpha$ CD20-IgG3-huIFN $\alpha$  Gly<sub>4</sub>Ser linker, nucleic acid (SEQ ID NO:9), amino acid sequence (SEQ ID NO:10); FIG. 1F:  $\alpha$ CD20-IgG3-huIFN $\alpha$  alpha helical linker, nucleic acid (SEQ ID NO:11), amino acid sequence (SEQ ID NO:12); FIG. 1G:  $\alpha$ CD20-IgG1-muIFN $\alpha$  Gly<sub>4</sub>Ser linker, nucleic acid (SEQ ID NO:13), amino acid sequence (SEQ ID NO:14); FIG. 1H:  $\alpha$ CD20-IgG1-muIFN $\alpha$  alpha helical linker, nucleic acid (SEQ ID NO:15), amino acid sequence (SEQ ID NO:16); FIG. 1I:  $\alpha$ CD20-IgG1-huIFN $\alpha$  Gly<sub>4</sub>Ser linker, nucleic acid (SEQ ID NO:17), amino acid sequence (SEQ ID NO:18); FIG. 1J:  $\alpha$ CD20-IgG1-huIFN $\alpha$  alpha helical linker, nucleic acid (SEQ ID NO:19), amino acid sequence (SEQ ID NO:20); FIG. 1K:  $\alpha$ Her2/neu light chain nucleic acid (SEQ ID NO:21), amino acid sequence (SEQ ID NO:22); FIG. 1L:  $\alpha$ Her2/neu-IgG1-muIFN $\alpha$  glyser linker nucleic acid sequence (SEQ ID NO:23), amino acid sequence (SEQ ID

NO:24); FIG. 1M:  $\alpha$ Her2/neu-IgG1-muIFN $\alpha$  alpha helical linker nucleic acid sequence (SEQ ID NO:25), amino acid sequence (SEQ ID NO:26); FIG. 1N:  $\alpha$ Her2/neu-IgG1-huIFN $\alpha$  glyser linker nucleic acid sequence (SEQ ID NO:27), amino acid sequence (SEQ ID NO:28); FIG. 1o:  $\alpha$ Her2/neu-IgG1-huIFN $\alpha$  alpha helical linker nucleic acid sequence (SEQ ID NO:29), amino acid sequence (SEQ ID NO:30). It will be appreciated that while the constructs in this figure are shown with particular linkers, in certain embodiments other linkers can be substituted therefore as described herein.

FIGS. 2A, 2B, 2C, and 2D illustrate the construction and characterization of anti-HER2/neu IgG3-IFN- $\alpha$ . FIG. 2A: Schematic diagram of anti-HER2/neu-IgG3-IFN- $\alpha$ . Solid areas represent anti-HER2/neu variable regions. Open areas represent human IgG3 and  $\kappa$  constant regions. White circle regions represent murine IFN- $\alpha$ . FIG. 2B: SDS-PAGE of purified anti-HER2/neu-IgG3 (lanes 1 and 4), IgG3-IFN- $\alpha$  (lanes 2 and 5), and anti-HER2/neu-IgG3-IFN- $\alpha$  (lanes 3 and 6) under nonreducing (lanes 1-3) or reducing (lanes 4-6) conditions. The molecular mass marker proteins are shown at the left of each gel. FIG. 2C: Anti-HER2/neu-IgG3 and anti-HER2/neu-IgG3-IFN- $\alpha$  bind HER2/neu. CT26/HER2, a murine colonic cell line expressing high levels of human HER2/neu, was reacted with anti-HER2/neu-IgG3, IgG3-IFN- $\alpha$ , or anti-HER2/neu-IgG3-IFN- $\alpha$  with or without heparin followed by PE-labeled rabbit anti-human IgG. Dashed lines represent signal from cells without addition of recombinant protein. FIG. 2D: The protective activity of the IFN- $\alpha$  standard and different IFN- $\alpha$  fusion proteins against VSV. Dilutions of 1 U of IFN- $\alpha$  standard, 0.21 ng (10 pM) of anti-HER2/neu-IgG3-IFN- $\alpha$ , 0.21 ng (10 pM) of IgG3-IFN- $\alpha$ , or 0.17 ng (10 pM) of anti-HER2/neu-IgG3 in 100  $\mu$ l were prepared and added to L-929 cells. After a 24-h incubation, 4000 PFU of VSV were added. Forty-eight hours later, viable cells were stained with crystal violet dye, dissolved by methanol, and solubilized dye was detected using an ELISA reader at 570 nm.

FIGS. 3A and 3B show in vivo antitumor activity of different IFN- $\alpha$  fusion proteins and rIFN- $\alpha$ . C3H/HeN mice were s.c. challenged with  $1 \times 10^3$  38C13/HER2 cells and i.p. treated with either 2.5  $\mu$ g (FIG. 3A) or 1  $\mu$ g (FIG. 3B) of the indicated proteins at days 1, 3, and 5 after tumor challenge. The tumor volume of each mouse is measured. Animals were observed until the diameter of the s.c. tumor reached 15 mm.

FIGS. 4A and 4B show that fusion of IgG3 to IFN- $\alpha$  improved its antitumor activity and increased its in vivo half-life. FIG. 4A: Mice were treated with 9600 U of rIFN- $\alpha$  or 9600 U (4  $\mu$ g) of IgG3-IFN- $\alpha$  at days 1 and 3 after tumor challenge. Animals were followed for survival and sacrificed when the diameter of the s.c. tumor reached 15 mm. FIG. 4B: Groups of three C3H/HeN mice were injected i.p. with 66  $\mu$ Ci of <sup>125</sup>I-labeled rIFN- $\alpha$ , IgG3-IFN- $\alpha$  or, anti-HER2/neu-IgG3-IFN- $\alpha$ . At various intervals after injection of the <sup>125</sup>I-labeled proteins, residual radioactivity was measured using a mouse whole body counter. The results represent the mean of three mice. Bars, SD.

FIGS. 5A, 5B, 5C, and 5D show that IFN- $\alpha$  fusion proteins inhibited cell proliferation and induced apoptosis in 38C13/HER2 cells in vitro. IFN- $\alpha$  fusion proteins inhibited tumor cell proliferation. After incubation for 48 h with different doses of the different fusion proteins, viable 38C13/HER2 (FIG. 5A) or 38C13 (FIG. 5B) cells were measured using the MTS assay. These experiments were performed three times in triplicate; error bars, SD of the measurements. FIG. 5C: IFN- $\alpha$  fusion proteins induce apoptosis in 38C13/HER2 cells. In brief,  $1 \times 10^6$  38C13/HER2 cells were incubated with

1 nM of the indicated proteins for 72 h. The cells were then washed, stained with Alexa Fluor 488, annexin V, and PI and were analyzed by flow cytometry. The percentage of cells located in each quadrant is indicated at the corner. FIG. 5D: IFN- $\alpha$  fusion proteins inhibited proliferation of surviving 38C13/HER2 cells. In brief,  $1 \times 10^6$  38C13/HER2 cells were labeled with 2.5  $\mu$ M CFSE and immediately fixed (dash line), or treated with PBS (thin black line), or 1 nM of either anti-HER2/neu IgG3 (thin black line, overlaps with PBS control), IgG3-IFN- $\alpha$  (thick black line), or anti-HER2/neu-IgG3-IFN- $\alpha$  (black area) for 48 h. The cells were then washed and analyzed by flow cytometry. The histogram was obtained by gating on the population of live cells.

FIGS. 6A, 6B, and 6C show that IFN- $\alpha$  fusion proteins induced STAT1 activation in 38C13/HER2 cells. In brief,  $1 \times 10^7$  38C13/HER2 cells were treated with 1000 U/ml of either anti-HER2/neu-IgG3-IFN- $\alpha$  (FIG. 6A) or IgG3-IFN- $\alpha$  (FIG. 6B) for the indicated times. The cell lysates were separated by SDS-PAGE and analyzed by Western blot using a polyclonal rabbit anti-phosphoSTAT1. To confirm equal loading of protein samples, blots were probed with a HRP-conjugated rabbit polyclonal Ab against GAPDH. FIG. 6C: The intensity of antiphosphoSTAT1 was normalized with the intensity of anti-GAPDH for each indicated time point, and the values obtained were divided by the value at time 0 to obtain the fold activation for STAT1. These experiments were performed twice; error bars, SD of the measurements. \*, Only point where the two groups differ with a p<0.05.

FIG. 7 IFN- $\alpha$  fusion proteins inhibit the growth of established tumor. C3H/HeN mice were injected s.c. with  $1 \times 10^3$  38C13/HER2 cells. After 12 days, mice were treated i.p. with 5  $\mu$ g of the indicated protein for 3 consecutive days. The tumor volume of each mouse is measured. Animals were sacrificed when the diameter of the s.c. tumor reached 15 mm.

FIG. 8 shows binding of recombinant antibodies to human cells expressing CD20. Daudi cells were incubated with either recombinant IgG3 or Rituximab followed by biotinylated rat anti-human IgG and PE-labeled streptavidin and analyzed by flow-cytometry. A, cells with only the secondary antibody; B, cells with recombinant IgG3; C, cells with Rituximab.

FIG. 9 shows a diagram of the heavy chain of the antibody-IFN- $\alpha$  fusion protein. In particular, the figure illustrates shortening of the (Gly<sub>4</sub>Ser)<sub>3</sub> (SEQ ID NO:31) to a Gly<sub>4</sub>Ser (SEQ ID NO:32) linker enables production of full-length αCD20-IgG3-mIFN $\alpha$ .

FIG. 10 shows SDS-PAGE analysis of fractions eluted from protein A Sepharose. Culture supernatants from cells expressing anti-CD-20-IgG3-IFNa with the (Gly<sub>4</sub>Ser)<sub>3</sub> (SEQ ID NO:31) linker were passed through the protein A Sepharose and the fusion protein bound prior to elution. A. Proteins were run without reduction. Lane 1, IgG3; Lanes 2-6, fractions eluted from protein A Sepharose. B. Proteins were reduced prior to analysis. Lane 2, IgG3; Lanes 3-7, fractions eluted from protein A Sepharose.

FIG. 11 shows SDS-PAGE analysis of proteins made by transient expression in HEK293T cells. Lane 1, anti-CD20-IgG3-huIFN $\alpha$  with extended (Gly<sub>4</sub>Ser)<sub>3</sub> (SEQ ID NO:31) linker; Lane 2, anti-CD20-IgG3 huIFN $\alpha$  with shortened Gly<sub>4</sub>Ser (SEQ ID NO:32) linker; Lane 3, anti-CD20-IgG3-muIFN $\alpha$  with extended (Gly<sub>4</sub>Ser)<sub>3</sub> (SEQ ID NO:31) linker; Lane 4, anti-CD20-IgG3-muIFN $\alpha$  with shortened Gly<sub>4</sub>Ser linker; Lane 5, anti-CD20 IgG3.

FIG. 12 was shows an analysis of protein binding to Daudi cells using FLOW cytometry.  $1 \times 10^6$  Daudi cells were stained with 1  $\mu$ g of fusion protein containing human IFN- $\alpha$  or Rituxan.

FIG. 13 shows an analysis of protein binding to 38C13/CD20 by FLOW cytometry.

FIG. 14. Daudi cells were incubated with various concentrations of IFN- $\alpha$ , antibody or fusion protein for 72 hrs. Growth inhibition was assessed using the CellTiter 96 AQueous cell proliferation assay.

FIG. 15. Daudi cells were treated with 10 pM of the indicated proteins for 72 hrs. Cell viability and apoptosis was determined following staining with Annexin V and PI and analysis by FLOW cytometry.

FIG. 16. 38C13/CD20 cells were treated with 10 pM of the indicated proteins for 48 hours. Cell viability and apoptosis was determined following staining with Annexin V and PI and analysis by FLOW cytometry.

FIG. 17. Inhibition of cell proliferation following treatment with different proteins at varying concentrations. 38C13-CD20 cells were treated with the indicated proteins at varying concentrations for 48 hours. After treatment the extent of proliferation was monitored using the MTS assay.

FIG. 18. 38C13/CD20 cells were treated with the different concentrations of the indicated proteins for 48 hours. Cell viability and apoptosis was determined following staining with Annexin V and PI and analysis by FLOW cytometry.

FIG. 19. Daudi cells were incubated for 72 hours with different concentrations of the fusion protein. Cell viability and apoptosis was determined following staining with Annexin V and PI and analysis by FLOW cytometry.

FIG. 20. Daudi cells were treated for 72 hours with various concentrations of fusion proteins. MTS solution was added to quantitate cell viability.

FIG. 21. Daudi cells were incubated with 72 hours with 1 pM of anti-CD20-IgG3-hIFN $\alpha$  with the Gly<sub>4</sub>Ser linker (32) (Gly-Ser Linker) or with 1 pM of anti-CD20-IgG3-hIFN $\alpha$  with the alpha helical linker (Alpha helix Linker). Cell viability and apoptosis was determined following staining with Annexin V and PI and analysis by FLOW cytometry.

FIG. 22 shows survival of mice inoculated with 5000 38C13-CD20 cells and treated on days 1, 2 and 3 with HBSS or the indicated amounts of the anti-CD20-IFN- $\alpha$  fusion proteins.

FIG. 23 shows survival of mice inoculated with 5000 38C13-CD20 cells and treated on days 5, 6 and 7 with 10  $\mu$ g of anti-CD20-IgG1, anti-CD20-IgG3, Rituximab or anti-CD20-IgG3-mIFN $\alpha$ .

FIG. 24. Survival of mice inoculated with 5000 38C13-CD20 cells and treated on days 5, 6 and 7 with 10  $\mu$ g of anti-CD20-IgG3, anti-CD20-IgG3 +IFNa, anti-DNS-IgG3, or anti-CD20-IgG3-mIFN $\alpha$ .

FIG. 25. Groups of eight mice were injected with 5000 38C13-CD20 cells on days 0. One days 8, 9 and 10 they were treated with HBSS or 100  $\mu$ g of anti-CD20-IgG3-mIFN $\alpha$ . Tumor growth was monitored over time.

FIG. 26. Groups of eight mice were injected with 5000 38C13-CD20 cells on days 0. One days 8, 9 and 10 they were treated with HBSS or 100  $\mu$ g of anti-CD20-IgG3-mIFN $\alpha$ . Survival was monitored over time.

## DETAILED DESCRIPTION

Interferon alpha (IFN- $\alpha$ ) is an important cytokine in initiating the innate immune response and also demonstrates a wide spectrum of anti-tumor activities. The clinical use of interferon (e.g., IFN- $\alpha$ ) as an anticancer drug, however, is hampered by its short half-life, which significantly compromises its therapeutic effect. In certain embodiments this invention pertains to the discovery that the therapeutic index of interferon can be improved by attaching the interferon to a

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targeting moiety that specifically/preferentially binds a marker on or associated with the target cell (e.g., a tumor cell). This permits the deliver of higher doses of interferon to the target site with fewer systemic complications. This was illustrated, in one embodiment, by the construction and use of a fusion protein consisting of an anti-HER2/neu IgG3 and IFN- $\alpha$  (anti-HER2/neu-IgG3-IFN- $\alpha$ ) and in another embodiment by the construction and use of an anti-CD20-IFN- $\alpha$  fusion protein.

The efficacy of the HER2/neu-IgG3-IFN- $\alpha$  constructs was tested on a murine B-cell lymphoma, 38C13, transduced with human HER2/neu. The anti-HER2/neu-IgG3-IFN- $\alpha$  fusion protein exhibited a potent effect in inhibiting the 38C13/HER2 tumor growth in vivo, and even administration of 1  $\mu$ g anti-HER2/neu IgG3-IFN- $\alpha$  resulted in 88% of long-term survivors after tumor challenge.

Remarkably, Anti-HER2/neu IgG3-IFN- $\alpha$  demonstrated a potent activity against established 38C13/HER2 tumors, and complete tumor remission was observed in 88% treated mice. This dramatic anti-tumor activity was mediated by IFN- $\alpha$  induced apoptosis and targeting IFN- $\alpha$  to 38C13/HER2 tumor cells by the anti-HER2/neu IgG3 antibody was essential to potentiate these effects.

Similar results were observed for the anti-CD20-IgG3-IFN- $\alpha$  construct (see, Example 2). These results indicate that attachment (e.g., fusion) of an interferon (e.g., IFN- $\alpha$ ) to a targeting moiety (e.g., to a tumor specific antibody) produces an effective therapeutic that can be used to inhibit the growth and/or proliferation or even to kill target cell(s). Thus, for example, the exemplary constructs described herein can readily be used for treatment of B cell lymphoma and other cancers in clinic.

Thus, in certain embodiments, this invention provides constructs (e.g., chimeric moieties) comprising an interferon (e.g., IFN- $\alpha$ ) attached to a targeting moiety (e.g., to an antibody that specifically binds a cancer specific marker on a cancer cell). The constructs include chemical conjugates as well as fusion proteins. Also provided are nucleic acids encoding the fusion proteins as well as cells transfected with the nucleic acids to express the fusion proteins. Also provided are methods of inhibiting growth and proliferation of cancer cells as well as kits comprising, e.g. the chimeric moieties described herein, for the treatment of various cancers.

#### I. Chimeric Constructs Comprising a Targeting Moiety Attached to an Interferon.

It was a surprising discovery that chimeric constructs comprising a targeting moiety (e.g., an anti-tumor marker antibody) attached to a native (wildtype) or modified IFN (e.g., IFN- $\alpha$ ) can be effectively used to inhibit the growth and/or proliferation of target cancer cells expressing or associated with the marker to which the targeting moiety is directed. In certain embodiments the targeting moieties are chemically conjugated to the interferon, while in other embodiments, the targeting moiety is expressed as a fusion protein with the IFN- $\alpha$ . When produced as a fusion protein the targeting moiety (e.g., antibody) component can be directly fused to the IFN- $\alpha$  or attached by means of a peptide linker (e.g., a (Gly<sub>4</sub>Ser)<sub>3</sub> (SEQ ID NO:31) linker, a GlyGlyGlySer (SEQ ID NO:32) linker, a AEAAAKEAAAKA (SEQ ID NO:33), and the like.

##### A) Targeting Moieties.

In various embodiments, the targeting moiety is a molecule that specifically or preferentially binds a marker expressed by (e.g., on the surface of) or associated with the target cell(s). While essentially any cell can be targeted, certain preferred cells include those associated with a pathology characterized by hyperproliferation of a cell (i.e., a hyperproliferative dis-

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order). Illustrative hyperproliferative disorders include, but are not limited to psoriasis, neutrophilia, polycythemia, thrombocytosis, and cancer.

Hyperproliferative disorders characterized as cancer include but are not limited to solid tumors, such as cancers of the breast, respiratory tract, brain, reproductive organs, digestive tract, urinary tract, eye, liver, skin, head and neck, thyroid, parathyroid and their distant metastases. These disorders also include lymphomas, sarcomas, and leukemias.

5 Examples of breast cancer include, but are not limited to invasive ductal carcinoma, invasive lobular carcinoma, ductal carcinoma in situ, and lobular carcinoma in situ. Examples of cancers of the respiratory tract include, but are not limited to small-cell and non-small-cell lung carcinoma, as well as bronchial adenoma and pleuropulmonary blastoma. Examples of brain cancers include, but are not limited to brain stem and hypothalamic glioma, cerebellar and cerebral astrocytoma, medulloblastoma, ependymoma, as well as neuroectodermal and pineal tumor. Tumors of the male reproductive organs include, but are not limited to prostate and testicular cancer. Tumors of the female reproductive organs include, but are not limited to endometrial, cervical, ovarian, vaginal, and vulvar cancer, as well as sarcoma of the uterus. Tumors of the digestive tract include, but are not limited to anal, colon, 10 colorectal, esophageal, gallbladder, gastric, pancreatic, rectal, small-intestine, and salivary gland cancers. Tumors of the urinary tract include, but are not limited to bladder, penile, kidney, renal pelvis, ureter, and urethral cancers. Eye cancers include, but are not limited to intraocular melanoma and 15 retinoblastoma. Examples of liver cancers include, but are not limited to hepatocellular carcinoma (liver cell carcinomas with or without fibrolamellar variant), cholangiocarcinoma (intrahepatic bile duct carcinoma), and mixed hepatocellular cholangiocarcinoma. Skin cancers include, but are not limited to squamous cell carcinoma, Kaposi's sarcoma, malignant melanoma, Merkel cell skin cancer, and non-melanoma skin cancer. Head-and-neck cancers include, but are not limited to laryngeal/hypopharyngeal/nasopharyngeal/oropharyngeal cancer, and lip and oral cavity cancer. Lymphomas 20 include, but are not limited to AIDS-related lymphoma, non-Hodgkin's lymphoma, cutaneous T-cell lymphoma, Hodgkin's disease, and lymphoma of the central nervous system. Sarcomas include, but are not limited to sarcoma of the soft tissue, osteosarcoma, malignant fibrous histiocytoma, 25 lymphosarcoma, and rhabdomyosarcoma. Leukemias include, but are not limited to acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, and hairy cell leukemia.

These disorders have been well characterized in humans, 30 but also exist with a similar etiology in other mammals, and can be treated by administering pharmaceutical compositions of the present invention.

In certain embodiments, the targeting moiety is a moiety that binds a cancer marker (e.g., a tumor associated antigen). 35 A wide variety of cancer markers are known to those of skill in the art. The markers need not be unique to cancer cells, but can also be effective where the expression of the marker is elevated in a cancer cell (as compared to normal healthy cells) or where the marker is not present at comparable levels in 40 surrounding tissues (especially where the chimeric moiety is delivered locally).

Illustrative cancer markers include, for example, the tumor marker recognized by the ND4 monoclonal antibody. This marker is found on poorly differentiated colorectal cancer, as 45 well as gastrointestinal neuroendocrine tumors (see, e.g., Tobi et al. (1998) *Cancer Detection and Prevention*, 22(2): 147-152). Other important targets for cancer immunotherapy

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are membrane bound complement regulatory glycoprotein: CD46, CD55 and CD59, which have been found to be expressed on most tumor cells in vivo and in vitro. Human mucins (e.g. MUC1) are known tumor markers as are gp100, tyrosinase, and MAGE, which are found in melanoma. Wild-type Wilms' tumor gene WT1 is expressed at high levels not only in most of acute myelocytic, acute lymphocytic, and chronic myelocytic leukemia, but also in various types of solid tumors including lung cancer.

Acute lymphocytic leukemia has been characterized by the TAAAs HLA-Dr, CD1, CD2, CD5, CD7, CD19, and CD20. Acute myelogenous leukemia has been characterized by the TAAAs HLA-Dr, CD7, CD13, CD14, CD15, CD33, and CD34. Breast cancer has been characterized by the markers EGFR, HER2, MUC1, Tag-72. Various carcinomas have been characterized by the markers MUC1, TAG-72, and CEA. Chronic lymphocytic leukemia has been characterized by the markers CD3, CD19, CD20, CD21, CD25, and HLA-DR. Hairy cell leukemia has been characterized by the markers CD19, CD20, CD21, CD25. Hodgkin's disease has been characterized by the Leu-M1 marker. Various melanomas have been characterized by the HMB 45 marker. Non-hodgkins lymphomas have been characterized by the CD20, CD19, and Ia marker. And various prostate cancers have been characterized by the PSMA and SE10 markers.

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In addition, many kinds of tumor cells display unusual antigens that are either inappropriate for the cell type and/or its environment, or are only normally present during the organisms' development (e.g. fetal antigens). Examples of such antigens include the glycosphingolipid GD2, a disialoganglioside that is normally only expressed at a significant level on the outer surface membranes of neuronal cells, where its exposure to the immune system is limited by the blood-brain barrier. GD2 is expressed on the surfaces of a wide range of tumor cells including neuroblastoma, medulloblastomas, astrocytomas, melanomas, small-cell lung cancer, osteosarcomas and other soft tissue sarcomas. GD2 is thus a convenient tumor-specific target for immunotherapies.

Other kinds of tumor cells display cell surface receptors that are rare or absent on the surfaces of healthy cells, and which are responsible for activating cellular signaling pathways that cause the unregulated growth and division of the tumor cell. Examples include (ErbB2). HER2/neu, a constitutively active cell surface receptor that is produced at abnormally high levels on the surface of breast cancer tumor cells.

Other useful targets include, but are not limited to CD20, CD52, CD33, epidermal growth factor receptor and the like. An illustrative, but not limiting list of suitable tumor markers is provided in Table 1. Antibodies to these and other cancer markers are known to those of skill in the art and can be obtained commercially or readily produced, e.g. using phage-display technology.

TABLE 1

Illustrative cancer markers and associated references, all of which are incorporated herein by reference for the purpose of identifying the referenced tumor markers.

Marker	Reference
5 alpha reductase	Délos et al. (1998) <i>Int J Cancer</i> , 75: 6 840-846
$\alpha$ -fetoprotein	Esteban et al. (1996) <i>Tumour Biol.</i> , 17(5): 299-305
AM-1	Harada et al. (1996) <i>Tohoku J Exp Med.</i> , 180(3): 273-288
APC	Dihlmann et al. (1997) <i>Oncol Res.</i> , 9(3) 119-127
APRIL	Sordat et al. (1998) <i>J Exp Med.</i> , 188(6): 1185-1190
BAGE	Böel et al. (1995) <i>Immunity</i> , 2: 167-175.
$\beta$ -catenin	Hugh et al. (1999) <i>Int J Cancer</i> , 82(4): 504-11
Be12	Koty et al. (1999) <i>Lung Cancer</i> , 23(2): 115-127
bcr-ab1 (b3a2)	Verfaillie et al. (1996) <i>Blood</i> , 87(11): 4770-4779
CA-125	Bast et al. (1998) <i>Int J Biol Markers</i> , 13(4): 179-187
CASP-8/FLICE	Mandruzzato et al. (1997) <i>J Exp Med.</i> , 186(5): 785-793.
Cathepsin	Thomassen et al. (1995) <i>Clin Cancer Res.</i> , 1(7): 741-746
CD19	Scheuermann et al. (1995) <i>Leuk Lymphoma</i> , 18(5-6): 385-397
CD20	Knox et al. (1996) <i>Clin Cancer Res.</i> , 2(3): 457-470
CD21, CD23	Shubinsky et al. (1997) <i>Leuk Lymphoma</i> , 25(5-6): 521-530
CD22, CD38	French et al. (1995) <i>Br J Cancer</i> , 71(5): 986-994
CD33	Nakase et al. (1996) <i>Am J Clin Pathol.</i> , 105(6): 761-768
CD35	Yamakawa et al. <i>Cancer</i> , 73(11): 2808-2817
CD44	Naot et al. (1997) <i>Adv Cancer Res.</i> , 71: 241-319
CD45	Buzzi et al. (1992) <i>Cancer Res.</i> , 52(14): 4027-4035
CD46	Yamakawa et al. (1994) <i>Cancer</i> , 73(11): 2808-2817
CD5	Stein et al. (1991) <i>Clin Exp Immunol.</i> , 85(3): 418-423
CD52	Ginaldi et al. (1998) <i>Leuk Res.</i> , 22(2): 185-191
CD55	Spendlove et al. (1999) <i>Cancer Res.</i> , 59: 2282-2286.
CD59 (791Tgp72)	Jarvis et al. (1997) <i>Int J Cancer</i> , 71(6): 1049-1055
CDC27	Wang et al. (1999) <i>Science</i> , 284(5418): 1351-1354
CDK4	Wölfel et al. (1995) <i>Science</i> , 269(5228): 1281-1284
CEA	Kass et al. (1999) <i>Cancer Res.</i> , 59(3): 676-683
c-myc	Watson et al. (1991) <i>Cancer Res.</i> , 51(15): 3996-4000
Cox-2	Tsujii et al. (1998) <i>Cell</i> , 93: 705-716
DCC	Gotley et al. (1996) <i>Oncogene</i> , 13(4): 787-795
DeR3	Pitti et al. (1998) <i>Nature</i> , 396: 699-703
E6/E7	Steller et al. (1996) <i>Cancer Res.</i> , 56(21): 5087-5091
EGFR	Yang et al. (1999) <i>Cancer Res.</i> , 59(6): 1236-1243.
EMBP	Shiina et al. (1996) <i>Prostate</i> , 29(3): 169-176.
Ena78	Arenberg et al. (1998) <i>J. Clin. Invest.</i> , 102: 465-472.
FGF8b and FGF8a	Dorkin et al. (1999) <i>Oncogene</i> , 18(17): 2755-2761
FLK-1/KDR	Annie and Fong (1999) <i>Cancer Res.</i> , 59: 99-106
Folic Acid Receptor	Dixon et al. (1992) <i>J Biol Chem.</i> , 267(33): 24140-72414
G250	Divgi et al. (1998) <i>Clin Cancer Res.</i> , 4(11): 2729-2739

Illustrative cancer markers and associated references, all of which are incorporated herein by reference for the purpose of identifying the referenced tumor markers.

Marker	Reference
GAGE-Family	De Backer et al. (1999) <i>Cancer Res.</i> , 59(13): 3157-3165
gastrin 17	Watson et al. (1995) <i>Int J Cancer</i> , 61(2): 233-240
Gastrin-releasing hormone (bombesin)	Wang et al. (1996) <i>Int J Cancer</i> , 68(4): 528-534
GD2/GD3/GM2	Wiesner and Sweeley (1995) <i>Int J Cancer</i> , 60(3): 294-299
GnRH	Bahk et al.(1998) <i>Urol Res.</i> , 26(4): 259-264
GnTV	Hengstler et al. (1998) <i>Recent Results Cancer Res.</i> , 154: 47-85
gp100/Pmel17	Wagner et al. (1997) <i>Cancer Immunol Immunother.</i> , 44(4): 239-247
gp-100-in4	Kirkin et al. (1998) <i>APMIS</i> , 106(7): 665-679
gp15	Maeurer et al.(1996) <i>Melanoma Res.</i> , 6(1): 11-24
gp75/TRP-1	Lewis et al.(1995) <i>Semin Cancer Biol.</i> , 6(6): 321-327
hCG	Hoermann et al. (1992) <i>Cancer Res.</i> , 52(6): 1520-1524
Heparanase	Vlodaysky et al. (1999) <i>Nat Med.</i> , 5(7): 793-802
Her2/neu	Lewis et al. (1995) <i>Semin Cancer Biol.</i> , 6(6): 321-327
Her3	
HMTV	Kahl et al.(1991) <i>Br J Cancer</i> , 63(4): 534-540
Hsp70	Jaattela et al. (1998) <i>EMBO J.</i> , 17(21): 6124-6134
hTERT (telomerase)	Vonderheide et al. (1999) <i>Immunity</i> , 10: 673-679. 1999.
IGFR1	Ellis et al. (1998) <i>Breast Cancer Res. Treat.</i> , 52: 175-184
IL-13R	Murata et al. (1997) <i>Biochem Biophys Res Commun.</i> , 238(1): 90-94
iNOS	Klotz et al. (1998) <i>Cancer</i> , 82(10): 1897-1903
Ki 67	Gerdes et al. (1983) <i>Int J Cancer</i> , 31: 13-20
KIAA0205	Guéguen et al. (1998) <i>J Immunol.</i> , 160(12): 6188-6194
K-ras, H-ras, N-ras	Abrams et al. (1996) <i>Semin Oncol.</i> , 23(1): 118-134
KSA (CO17-1A)	Zhang et al. (1998) <i>Clin Cancer Res.</i> , 4(2): 295-302
LDLR-FUT	Caruso et al. (1998) <i>Oncol Rep.</i> , 5(4): 927-930
MAGE Family (MAGE1, MAGE3, etc.)	Marchand et al. (1999) <i>Int J Cancer</i> , 80(2): 219-230
Mammaglobin	Watson et al. (1999) <i>Cancer Res.</i> , 59: 13 3028-3031
MAP17	Kocher et al. (1996) <i>Am J Pathol</i> , 149(2): 493-500
Melan-A/ MART-1	Lewis and Houghton (1995) <i>Semin Cancer Biol.</i> , 6(6): 321-327
mesothelin	Chang et al. (1996) <i>Proc. Natl. Acad. Sci., USA</i> , 93(1): 136-140
MIC A/B	Groh et al.(1998) <i>Science</i> , 279: 1737-1740
MT-MMP's, such as MMP2, MMP3, MMP7, MMP9	Sato and Seiki (1996) <i>J Biochem (Tokyo)</i> , 119(2): 209-215
Mox1	Candia et al. (1992) <i>Development</i> , 116(4): 1123-1136
Mucin, such as MUC- 1, MUC-2, MUC-3, and MUC-4	Lewis and Houghton (1995) <i>Semin Cancer Biol.</i> , 6(6): 321-327
MUM-1	Kirkin et al. (1998) <i>APMIS</i> , 106(7): 665-679
NY-ESO-1	Jager et al. (1998) <i>J. Exp. Med.</i> , 187: 265-270
Osteonectin	Graham et al. (1997) <i>Eur J Cancer</i> , 33(10): 1654-1660
p15	Yoshida et al. (1995) <i>Cancer Res.</i> , 55(13): 2756-2760
P170/MDR1	Trock et al. (1997) <i>J Natl Cancer Inst.</i> , 89(13): 917-931
p53	Roth et al. (1996) <i>Proc. Natl. Acad. Sci., USA</i> , 93(10): 4781-4786.
p97/melanotransferrin	Furukawa et al. (1989) <i>J Exp Med.</i> , 169(2): 585-590
PAI-1	Gréndahl-Hansen et al. (1993) <i>Cancer Res.</i> , 53(11): 2513-2521
PDGF	Vassbotn et al. (1993) <i>Mol Cell Biol.</i> , 13(7): 4066-4076
Plasminogen (uPA)	Naitoh et al. (1995) <i>Jpn J Cancer Res.</i> , 86(1): 48-56
PRAME	Kirkin et al. (1998) <i>APMIS</i> , 106(7): 665-679
Probasin	Matuo et al. (1985) <i>Biochem Biophys Res Commun.</i> , 130(1): 293-300
Progenipoitin	—
PSA	Sanda et al. (1999) <i>Urology</i> , 53(2): 260-266.
PSM	Kawakami et.al.(1997) <i>Cancer Res.</i> , 57(12): 2321-2324
RAGE-1	Gaugler et al. (1996) <i>Immunogenetics</i> , 44(5): 323-330
Rb	Dosaka-Akita et al. (1997) <i>Cancer</i> , 79(7): 1329-1337
RCAS1	Sonoda et al.(1996) <i>Cancer</i> , 77(8): 1501-1509.
SART-1	Kikuchi et al.(1999) <i>Int J Cancer</i> , 81(3): 459-466
SSX gene family	Gure et al. (1997) <i>Int J Cancer</i> , 72(6): 965-971
STAT3	Bromberg et al. (1999) <i>Cell</i> , 98(3): 295-303
STn (mucin assoc.)	Sandmaier et al. (1999) <i>J Immunother.</i> , 22(1): 54-66
TAG-72	Kuroki et al. (1990) <i>Cancer Res.</i> , 50(16): 4872-4879
TGF-α	Imanishi et al. (1989) <i>Br J Cancer</i> , 59(5): 761-765
TGF-β	Picon et al. (1998) <i>Cancer Epidemiol Biomarkers Prev</i> , 7(6): 497-504
Thymosin β 15	Bao et al. (1996) <i>Nature Medicine</i> , 2(12), 1322-1328
IFN-α	Moradi et al. (1993) <i>Cancer</i> , 72(8): 2433-2440

TABLE 1-continued

Illustrative cancer markers and associated references, all of which are incorporated herein by reference for the purpose of identifying the referenced tumor markers.

Marker	Reference
TPA	Maulard et al. (1994) <i>Cancer</i> , 73(2): 394-398
TPI	Nishida et al. (1984) <i>Cancer Res</i> 44(8): 3324-9
TRP-2	Parkhurst et al. (1998) <i>Cancer Res.</i> , 58(21) 4895-4901
Tyrosinase	Kirklin et al. (1998) <i>APMIS</i> , 106(7): 665-679
VEGF	Hyodo et al. (1998) <i>Eur J Cancer</i> , 34(13): 2041-2045
ZAG	Sanchez et al. (1999) <i>Science</i> , 283(5409): 1914-1919
p16INK4	Quelle et al. (1995) <i>Oncogene</i> Aug. 17, 1995; 11(4): 635-645
Glutathione S-transferase	Hengstler (1998) et al. <i>Recent Results Cancer Res.</i> , 154: 47-85

Any of the foregoing markers can be used as targets for the targeting moieties comprising the interferon-targeting moiety constructs of this invention. In certain embodiments the target markers include, but are not limited to members of the epidermal growth factor family (e.g., HER2, HER3, EGF, HER4), CD1, CD2, CD3, CD5, CD7, CD13, CD14, CD15, CD19, CD20, CD21, CD23, CD25, CD33, CD34, CD38, 5E10, CEA, HLA-DR, HM 1.24, HMB 45, 1a, Leu-M1, MUC1, PMSA, TAG-72, phosphatidyl serine antigen, and the like.

The foregoing markers are intended to be illustrative and not limiting. Other tumor associated antigens will be known to those of skill in the art.

Where the tumor marker is a cell surface receptor, ligand to that receptor can function as targeting moieties. Similarly mimetics of such ligands can also be used as targeting moieties.

#### Antibodies.

In certain embodiments, the targeting moieties can comprise antibodies, unibodies, or affybodies that specifically or preferentially bind the tumor marker. Antibodies that specifically or preferentially bind tumor markers are well known to those of skill in the art. Thus, for example, antibodies that bind the CD22 antigen expressed on human B cells include HD6, RFB4, UV22-2, To15, 4KB128, a humanized anti-CD22 antibody (hLL2) (see, e.g., Li et al. (1989) *Cell. Immunol.* 111: 85-99; Mason et al. (1987) *Blood* 69: 836-40; Behr et al. (1999) *Clin. Cancer Res.* 5: 3304s-3314s; Bonardi et al. (1993) *Cancer Res.* 53: 3015-3021).

Antibodies to CD33 include for example, HuM195 (see, e.g., Kossman et al. (1999) *Clin. Cancer Res.* 5: 2748-2755), CMA-676 (see, e.g., Sievers et al., (1999) *Blood* 93: 3678-3684.

Antibodies to CD38 include for example, AT13/5 (see, e.g., Ellis et al. (1995) *J. Immunol.* 155: 925-937), HB7, and the like.

In certain embodiments the targeting moiety comprises an anti-HER2 antibody. The ergB 2 gene, more commonly known as (Her-2/neu), is an oncogene encoding a transmembrane receptor. Several antibodies have been developed against Her-2/neu, including trastuzumab (e.g., HERCEPTIN®.; Fornier et al. (1999) *Oncology (Huntingt)* 13: 647-58), TAB-250 (Rosenblum et al. (1999) *Clin. Cancer Res.* 5: 865-874), BACH-250 (Id.), TA1 (Maier et al. (1991) *Cancer Res.* 51: 5361-5369), and the mAbs described in U.S. Pat. Nos. 5,772,997; 5,770,195 (mAb 4D5; ATCC CRL 10463); and U.S. Pat. No. 5,677,171.

Illustrative anti-MUC-1 antibodies include, but are not limited to Mc5 (see, e.g., Peterson et al. (1997) *Cancer Res.* 57: 1103-1108; Ozzello et al. (1993) *Breast Cancer Res.*

*Treat.* 25: 265-276), and hCTMO1 (see, e.g., Van Hof et al. (1996) *Cancer Res.* 56: 5179-5185).

20 Illustrative anti-TAG-72 antibodies include, but are not limited to CC49 (see, e.g., Pavlinkova et al. (1999) *Clin. Cancer Res.* 5: 2613-2619), B72.3 (see, e.g., Divgi et al. (1994) *Nucl. Med. Biol.* 21: 9-15), and those disclosed in U.S. Pat. No. 5,976,531.

25 Illustrative anti-HM1.24 antibodies include, but are not limited to a mouse monoclonal anti-HM1.24 IgG<sub>2a</sub>/κ and a humanized anti-HM1.24 IgG<sub>1</sub>/κ. antibody (see, e.g., Ono et al. (1999) *Mol. Immuno.* 36: 387-395).

30 A number of antibodies have been developed that specifically bind HER2 and some are in clinical use. These include, for example, trastuzumab (e.g., HERCEPTIN®, Fornier et al. (1999) *Oncology (Huntingt)* 13: 647-658), TAB-250 (Rosenblum et al. (1999) *Clin. Cancer Res.* 5: 865-874), BACH-250 (Id.), TA1 (see, e.g., Maier et al. (1991) *Cancer Res.* 51: 5361-5369), and the antibodies described in U.S. Pat. Nos. 5,772,997; 5,770,195, and 5,677,171.

35 Other fully human anti-HER2/neu antibodies are well known to those of skill in the art. Such antibodies include, but are not limited to the C6 antibodies such as C6.5, DPL.5, G98A, C6MH3-B1, B1D2, C6VLD, C6VLE, C6VLF, C6MH3-D7, C6MH3-D6, C6MH3-D5, C6MH3-D3, C6MH3-D2, C6MH3-D1, C6MH3-C4, C6MH3-C3, C6MH3-B9, C6MH3-B5, C6MH3-B48, C6MH3-B47, C6MH3-B46, C6MH3-B43, C6MH3-B41, C6MH3-B39, C6MH3-B34, C6MH3-B33, C6MH3-B31, C6MH3-B27, C6MH3-B25, C6MH3-B21, C6MH3-B20, C6MH3-B2, C6MH3-B16, C6MH3-B15, C6MH3-B11, C6MH3-B1, C6MH3-A3, C6MH3-A2, and C6ML3-9. These and other anti-HER2/neu antibodies are described in U.S. Pat. Nos. 6,512,097 and 5,977,322, in PCT Publication WO 97/00271, 50 in Schier et al. (1996) *J Mol Biol* 255: 28-43, Schier et al. (1996) *J Mol Biol* 263: 551-567, and the like.

More generally, antibodies directed to various members of the epidermal growth factor receptor family are well suited for use as targeting moieties in the constructs of the present invention. Such antibodies include, but are not limited to anti-EGF-R antibodies as described in U.S. Pat. Nos. 5,844,093 and 5,558,864, and in European Patent No. 706,799A.). Other illustrative anti-EGFR family antibodies include, but are not limited to antibodies such as C6.5, C6ML3-9, C6MH3-B1, C6-B1D2, F5, HER3.A5, HER3.F4, HER3.H1, HER3.I3, HER3.E12, HER3.B12, EGFR.E12, EGFR.C10, EGFR.B11, EGFR.E8, HER4.B4, HER4.G4, HER4.F4, HER4.A8, HER4.B6, HER4.D4, HER4.D7, HER4.D11, HER4.D12, HER4.E3, HER4.E7, HER4.F8 and HER4.C7 and the like (see, e.g., U.S. Patent publications US 2006/0099205 A1 and US 2004/0071696 A1 which are incorporated herein by reference).

As described in U.S. Pat. Nos. 6,512,097 and 5,977,322 other anti-EGFR family member antibodies can readily be produced by shuffling light and/or heavy chains followed by one or more rounds of affinity selection. Thus in certain embodiments, this invention contemplates the use of one, two, or three CDRs in the VL and/or VH region that are CDRs described in the above-identified antibodies and/or the above identified publications.

In various embodiments the targeting moiety comprises an antibody that specifically or preferentially binds CD20. Anti-CD20 antibodies are well known to those of skill and include, but are not limited to rituximab, Ibrutinomab tiuxetan, and tositumomab, AME-133v (Applied Molecular Evolution), Ocrelizumab (Roche), Ofatumumab (Genmab), TRU-015 (Trubion) and IMMU-106 (Immunomedics).

The invention need not be limited to the use of the antibodies described above, and other such antibodies as they are known to those of skill in the art can be used in the compositions and methods described herein.

While the above discussion pertains to antibodies, it will be recognized that affibodies and/or unibodies can be used instead of antibodies.

#### Unibodies.

UniBody are antibody technology that produces a stable, smaller antibody format with an anticipated longer therapeutic window than certain small antibody formats. In certain embodiments unibodies are produced from IgG4 antibodies by eliminating the hinge region of the antibody. Unlike the full size IgG4 antibody, the half molecule fragment is very stable and is termed a uniBody. Halving the IgG4 molecule left only one area on the UniBody that can bind to a target. Methods of producing unibodies are described in detail in PCT Publication WO2007/059782, which is incorporated herein by reference in its entirety (see, also, Kolfschoten et al. (2007) *Science* 317: 1554-1557).

#### Affibodies.

Affibody molecules are class of affinity proteins based on a 58-amino acid residue protein domain, derived from one of the IgG-binding domains of *staphylococcal* protein A. This three helix bundle domain has been used as a scaffold for the construction of combinatorial phagemid libraries, from which Affibody variants that target the desired molecules can be selected using phage display technology (see, e.g., Nord et al. (1997) *Nat. Biotechnol.* 15: 772-777; Ronmark et al. (2002) *Eur. J. Biochem.*, 269: 2647-2655.). Details of Affibodies and methods of production are known to those of skill (see, e.g., U.S. Pat. No. 5,831,012 which is incorporated herein by reference in its entirety).

It will be recognized that the antibodies described above can be provided as whole intact antibodies (e.g., IgG), antibody fragments, or single chain antibodies, using methods well known to those of skill in the art. In addition, while the antibody can be from essentially any mammalian species, to reduce immunogenicity, it is desirable to use an antibody that is of the species in which the construct (e.g., anti-HER2/neu-IFN- $\alpha$  chimera) is to be used. In other words, for use in a human, it is desirable to use a human, humanized, or chimeric human antibody.

#### B) IFN- $\alpha$ and Modified IFN- $\alpha$

In various embodiments chimeric moieties of this invention comprise an interferon (e.g., IFN- $\alpha$ ) joined to the targeting moiety (e.g., anti-HER2/neu antibody). The interferon can be a full length wild-type interferon (e.g., IFN- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$ , etc.) an interferon fragment (e.g., an IFN- $\alpha$  fragment), and/or a mutated interferon. Typically the interferon fragment is one that possesses the endogenous activity of preferably at

a level of at least 80%, more preferably at least 90% or 95%, most preferably at least 98%, 99%, 100%, or a level greater than the wild-type interferon.

Means of identifying such modified interferon molecules are routine to those of skill in the art. In one illustrative approach, a library of truncated and/or mutated IFN- $\alpha$  is produced and screened for IFN- $\alpha$  activity. Methods of producing libraries of polypeptide variants are well known to those of skill in the art. Thus, for example error-prone PCR can be used to create a library of mutant and/or truncated IFN- $\alpha$  (see, e.g., U.S. Pat. No. 6,365,408).

The resulting library members can then be screened according to standard methods known to those of skill in the art. Thus, for example, IFN- $\alpha$  activity can be assayed by measuring antiviral activity against a particular test virus. Kits for assaying for IFN- $\alpha$  activity are commercially available (see, e.g., iLite<sup>TM</sup> alphabeta kit by Neutekbio, Ireland).

These methods are intended to be illustrative and not limiting. Using the teaching provided herein, other suitable modified interferons (e.g., modified IFN- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$ , etc.) can readily be identified and produced.

#### C. Attachment of the Antibody (e.g., Anti-HER2/neu) to the IFN- $\alpha$ .

Generally speaking, the targeting moiety (e.g., an anti-HER2/neu antibody, and anti-CD20 antibody, etc.) can be joined together in any order. Thus, for example, the antibody can be joined to either the amino or carboxy terminal of the interferon. The antibody can also be joined to an internal region of the interferon, or conversely, the interferon can be joined to an internal location or to any terminus of the antibody, as long as the attachment does not interfere with binding of the antibody to that target marker (e.g., the HER2/neu receptor).

The antibody (e.g., a C6 anti-HER2/neu) and the interferon (e.g., IFN- $\alpha$ ) can be attached by any of a number of means well known to those of skill in the art. In certain embodiments, the interferon is conjugated, either directly or through a linker (spacer), to the antibody. In certain embodiments, however, it is preferable to recombinantly express the chimeric moiety as a fusion protein.

##### i) Chemical Conjugation of the Targeting Moiety to the Interferon.

In certain embodiments, the targeting moiety (e.g., an anti-HER2/neu antibody such as C6.5, C6MH3-B1, G98A, ML3-9, H3B1, B1D2, etc.) is chemically conjugated to the interferon (e.g., IFN- $\alpha$ ) molecule. Means of chemically conjugating molecules are well known to those of skill

The procedure for conjugating two molecules varies according to the chemical structure of the agent. Polypeptides typically contain variety of functional groups; e.g., carboxylic acid (COOH) or free amine ( $-\text{NH}_2$ ) groups, that are available for reaction with a suitable functional group on the other peptide, or on a linker to join the molecules thereto.

Alternatively, the antibody and/or the IFN- $\alpha$  can be derivatized to expose or attach additional reactive functional groups. The derivatization can involve attachment of any of a number of linker molecules such as those available from Pierce Chemical Company, Rockford Ill.

A "linker", as used herein, typically refers to a molecule that is used to join the antibody to the IFN- $\alpha$ . In various embodiments, the linker is capable of forming covalent bonds to both the antibody and to the IFN- $\alpha$ . Suitable linkers are well known to those of skill in the art and include, but are not limited to, straight or branched-chain carbon linkers, heterocyclic carbon linkers, or peptide linkers. In certain embodiments, the linker(s) can be joined to the constituent amino acids of the antibody and/or the IFN- $\alpha$  through their side

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groups (e.g., through a disulfide linkage to cysteine). In certain preferred embodiments, the linkers are joined to the alpha carbon amino and/or carboxyl groups of the terminal amino acids of the antibody and/or the IFN- $\alpha$ .

A bifunctional linker having one functional group reactive with a group on the antibody and another group reactive on the IFN- $\alpha$ , can be used to form the desired conjugate. Alternatively, derivatization can involve chemical treatment of the targeting moiety. Procedures for generation of, for example, free sulfhydryl groups on polypeptides, such as antibodies or antibody fragments, are known (See U.S. Pat. No. 4,659,839).

Many procedures and linker molecules for attachment of various compounds including radionuclide metal chelates, toxins and drugs to proteins such as antibodies are known. See, for example, European Patent Application No. 188,256; U.S. Pat. Nos. 4,671,958, 4,659,839, 4,414,148, 4,699,784; 4,680,338; 4,569,789; and 4,589,071; and Borlinghaus et al. (1987) *Cancer Res.* 47: 4071-4075. In particular, production of various immunotoxins is well-known within the art and can be found, for example in "Monoclonal Antibody-Toxin Conjugates: Aiming the Magic Bullet," Thorpe et al., *Monoclonal Antibodies in Clinical Medicine*, Academic Press, pp. 168-190 (1982); Waldmann (1991) *Science*, 252: 1657; U.S. Pat. Nos. 4,545,985 and 4,894,443, and the like.

## ii) Production of Fusion Proteins.

In certain embodiments, a chimeric targeting moiety-interferon fusion protein is synthesized using recombinant DNA methodology. Generally this involves creating a DNA sequence that encodes the fusion protein, placing the DNA in an expression cassette under the control of a particular promoter, expressing the protein in a host, isolating the expressed protein and, if required, renaturing the protein.

DNA encoding the fusion proteins (e.g. anti-HER2/neu-IFN- $\alpha$ , anti-CD20-IFN- $\alpha$ , etc.) of this invention can be prepared by any suitable method, including, for example, cloning and restriction of appropriate sequences or direct chemical synthesis by methods such as the phosphotriester method of Narang et al. (1979) *Meth. Enzymol.* 68: 90-99; the phosphodiester method of Brown et al. (1979) *Meth. Enzymol.* 68: 109-151; the diethylphosphoramide method of Beaucage et al. (1981) *Tetra. Lett.*, 22: 1859-1862); the solid support method of U.S. Pat. No. 4,458,066, and the like.

Chemical synthesis produces a single stranded oligonucleotide. This can be converted into double stranded DNA by hybridization with a complementary sequence, or by polymerization with a DNA polymerase using the single strand as a template. One of skill would recognize that while chemical synthesis of DNA is limited to sequences of about 100 bases, longer sequences may be obtained by the ligation of shorter sequences.

Alternatively, subsequences can be cloned and the appropriate subsequences cleaved using appropriate restriction enzymes. The fragments can then be ligated to produce the desired DNA sequence.

In certain embodiments, DNA encoding fusion proteins of the present invention can be cloned using DNA amplification methods such as polymerase chain reaction (PCR). Thus, for example, the gene for IFN- $\alpha$  is PCR amplified, using a sense primer containing the restriction site for, e.g., NdeI and an antisense primer containing the restriction site for HindIII. This can produce a nucleic acid encoding the mature IFN- $\alpha$  sequence and having terminal restriction sites. An antibody having "complementary" restriction sites can similarly be cloned and then ligated to the IFN- $\alpha$  and/or to a linker attached to the IFN- $\alpha$ . Ligation of the nucleic acid sequences and insertion into a vector produces a vector encoding IFN- $\alpha$  joined to the anti-HER2/neu antibody.

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While the two molecules can be directly joined together, one of skill will appreciate that the molecules can be separated by a peptide spacer consisting of one or more amino acids. Generally the spacer will have no specific biological activity other than to join the proteins or to preserve some minimum distance or other spatial relationship between them. In certain embodiments, however, the constituent amino acids of the spacer can be selected to influence some property of the molecule such as the folding, net charge, or hydrophobicity.

It was a surprising discovery, however, that certain linkers are unsuitable for preparation of fusion proteins of the present invention. Thus, for example, the (Gly<sub>4</sub>Ser)<sub>3</sub> (SEQ ID NO:31) linker was not well suited for the production of an anti-CD20-IFN- $\alpha$  construct. Without being bound to a particular theory, it is believed the interferon was being removed from the fusion protein by proteolysis. Western blot analysis using anti-Fc and anti-interferon, confirmed that both of the upper bands were heavy chains, but only the largest contained interferon.

Accordingly, in certain preferred embodiments, it is desirable to use a linker that is resistant to proteolysis. Certain preferred linkers are linkers that are not the (Gly<sub>4</sub>Ser)<sub>3</sub> (SEQ ID NO:31) linker. Certain preferred linkers are linkers shorter than 15 amino acids, or linkers shorter than 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, or 2 amino acids in length. In certain embodiments the linker is an alpha helical linker ranging in length up to about 12 or 13 or 14 amino acids in length.

Certain illustrative proteolysis-resistant linkers well suited for use in the constructs of this invention are shown in Table 2.

TABLE 2

<u>Illustrative proteolysis-resistant linkers</u>	
Linker Seq	SEQ ID NO
GGGS	32
A(EAAA) <sub>n</sub> A where n = 1	33
where n = 2	34
where n = 3	35
where n = 4	36
where n = 5	37
GGGG	38
GGGGGGG	39
GGAGG	40
GAGAGAGAGA	41
RPLSYRPPPFGPPSVRP	42
YPRSIYIRRHPSPSLTT	43
TPSHLSHILPSFGLPTFN	44
RPVSPFTPPRLSNSWLPA	45
SPAHHFPRSIIPRGPIRT	46
APGPSAPSHRSILPSRAFG	47
PRNSIHFLHPLLVAAPLGA	48
MPSLSGVLQVRYLSPPD	49
SPQYPSPLTLTLPHPHSPL	50
NPSLNPPSYLHRAPSRI	51

TABLE 2 -continued

Illustrative proteolysis-resistant linkers.	
Linker Seq	SEQ ID NO
LPWRTSLLPSLPLRRRP	52
PPLFAKGPVGLLRSFPP	53
VPPAPVVSLRSAHARPPY	54
LRPTPPRVRSYTCCPTP	55
PNVAHVLPPLTVPWDNLR	56
CNPLLPLCARSPAVRTFP	57

The nucleic acid sequences encoding the fusion proteins can be expressed in a variety of host cells, including *E. coli*, other bacterial hosts, yeast, and various higher eukaryotic cells such as the COS, CHO and HeLa cells lines and myeloma cell lines. The recombinant protein gene is typically operably linked to appropriate expression control sequences for each host. For *E. coli* this includes a promoter such as the T7, trp, or lambda promoters, a ribosome binding site and preferably a transcription termination signal. For eukaryotic cells, the control sequences will include a promoter and preferably an enhancer derived from immunoglobulin genes, SV40, cytomegalovirus, etc., and a polyadenylation sequence, and may include splice donor and acceptor sequences.

The plasmids of the invention can be transferred into the chosen host cell by well-known methods such as calcium chloride transformation for *E. coli* and calcium phosphate treatment or electroporation for mammalian cells. Cells transformed by the plasmids can be selected by resistance to antibiotics conferred by genes contained on the plasmids, such as the amp, gpt, neo and hyg genes.

Once expressed, the recombinant fusion proteins can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like (see, generally, R. Scopes (1982) *Protein Purification*, Springer-Verlag, N.Y.; Deutscher (1990) *Methods in Enzymology* Vol. 182: *Guide to Protein Purification*., Academic Press, Inc. N.Y., and the like). Substantially pure compositions of at least about 90 to 95% homogeneity are preferred, and 98 to 99% or more homogeneity are most preferred for pharmaceutical uses. Once purified, partially or to homogeneity as desired, the polypeptides may then be used therapeutically.

One of skill in the art would recognize that after chemical synthesis, biological expression, or purification, the fusion protein (e.g., anti-HER2/neu-IFN- $\alpha$ , anti-CD20-IFN- $\alpha$ , etc.) may possess a conformation substantially different than the native conformations of the constituent polypeptides. In this case, it may be necessary to denature and reduce the polypeptide and then to cause the polypeptide to re-fold into the preferred conformation. Methods of reducing and denaturing proteins and inducing re-folding are well known to those of skill in the art (see, e.g., Debinski et al. (1993) *J. Biol. Chem.*, 268: 14065-14070; Kreitman and Pastan (1993) *Bioconjug. Chem.*, 4: 581-585; and Buchner, et al. (1992) *Anal. Biochem.*, 205: 263-270). Debinski et al., for example, describe the denaturation and reduction of inclusion body proteins in guanidine-DTE. The protein is then refolded in a redox buffer containing oxidized glutathione and L-arginine.

In certain embodiments a transient expression system can be used to express the chimeric constructs described herein.

Although many cell lines potentially can be used, one cell line that works well for transient expression is 293T. For transient expression of 293T on Day 0, 9 million cells in 25 ml are seeded for each 150 mm tissue culture plate. A 1 mg/ml of PEI (Polyethylenimine) is made using sterile water. For the expression of a complete antibody or antibody fusion protein, 25  $\mu$ g each of H and L (50  $\mu$ g total) is used per plate. A volume of 5 ml is used for transfection of each 150 mm plate. The DNA is mixed with DMEM, the PEI is then added and the mixture is incubated at room temperature for 10 mins. 1.75  $\mu$ g PEI is used for each  $\mu$ g of DNA. For transfection, the old medium is removed, discarded and replaced with 20 ml of fresh medium (Iscoves+5% calf serum). The transfection mix is added and the plate is swirled. On Day 2, the medium is replaced with 30 ml of Iscoves medium containing 1% FBS (fetal bovine serum) to minimize the amount of bovine Ig present. Supernatants are collected from the cells on Days 4, 6 and 13 by removing the medium and replacing it with 30 ml of fresh Iscover containing 1% FBS.

The cloning and expression of an anti-HER2/neu-IFN- $\alpha$  fusion protein is illustrated herein in Example 1, while the cloning and expression of an anti-CD20-IFN- $\alpha$  fusion protein is shown in Example 2.

One of skill would recognize these expression methods are illustrative and not limiting. Modifications can be made to the fusion proteins described herein without diminishing their activity/efficacy. Some modifications may be made to facilitate the cloning, expression, or incorporation of the targeting molecule into a fusion protein. Such modifications are well known to those of skill in the art and include, for example, a methionine added at the amino terminus to provide an initiation site, or additional amino acids placed on either terminus to create conveniently located restriction sites or termination codons.

Other modifications can be made to increase serum half-life and/or bioavailability. Such modifications include, but are not limited to the incorporation of D amino acids (especially in the linker), the use of non-naturally occurring amino acids, pegylation of the fusion protein, and the like.

#### D. Other Multi-Valent Targeting Moieties.

In certain embodiments this invention contemplates the use of multivalent, preferably trivalent, quadrivalent, pentavalent or greater targeting moieties (e.g., anti-HER2/neu antibodies, anti-CD20 antibodies, etc.) to target the interferon to a target cell.

For example, multivalent anti-HER2/neu moieties can be produced by any of a number of methods. For example, linkers having three, four, or more reactive sites can be reacted with anti-HER2/neu antibodies to form a trimer or greater conjugate.

In certain embodiments, phage display, yeast display, bacterial display, or other display systems can be used to express and display multiple copies (e.g., at least 3, at least 4, at least 5, at least 6 copies, etc.) of a targeting (e.g., anti-HER2/neu, anti-CD20, etc.) antibody and thereby effectively provide a multivalent targeting moiety.

#### II. Combined Uses.

The chimeric constructs of this invention are useful for inhibiting the growth and/or proliferation of target cells (e.g., cancer cells). In various embodiments the chimeric moieties can be used to inhibit disease progression, to shrink tumor size, and/or to stabilize regression/remission.

Particularly in the treatment of cancer, the compositions and methods of the invention may also include additional therapeutic and/or pharmacologically acceptable agents. For instance, the compositions or methods may involve other agents for the treatment of cancer. Such agents include, but

are not limited to alkylating agents (e.g., mechlorethamine (Mustargen), cyclophosphamide (Cytoxan, Neosar), ifosfamide (Ifex), phenylalanine mustard; melphalen (Alkeran), chlorambucol (Leukeran), uracil mustard, estramustine (Emcyt), thiotepa (Thioplex), busulfan (Myleran), lomustine (CeeNU), carmustine (BiCNU, BCNU), streptozocin (Zanosar), dacarbazine (DTIC-Dome), cis-platinum, cisplatin (Platinol, Platinol AQ), carboplatin (Paraplatin), altretamine (Hexalen), etc.), antimetabolites (e.g. methotrexate (Amethopterin, Folex, Mexate, Rheumatrex), 5-fluorouracil (Adrucil, Efdex, Fluoroplex), floxuridine, 5-fluorodeoxyuridine (FUDR), capecitabine (Xeloda), fludarabine: (Fludara), cytosine arabinoside (Cytarabine, Cytosar, ARA-C), 6-mercaptopurine (Purinethol), 6-thioguanine (Thioguanine), gemcitabine (Gemzar), cladribine (Leustatin), deoxycoformycin; pentostatin (Nipent), etc.), antibiotics (e.g. doxorubicin (Adriamycin, Rubex, Doxil, Daunoxome-liposomal preparation), daunorubicin (Daunomycin, Cerubidine), idarubicin (Idamycin), valrubicin (Valstar), mitoxantrone (Novantrone), dactinomycin (Actinomycin D, Cosmegen), mithramycin, plicamycin (Mithracin), mitomycin C (Mutamycin), bleomycin (Blenoxane), procarbazine (Matulane), etc.), mitotic inhibitors (e.g. paclitaxel (Taxol), docetaxel (Taxotere), vinblastine sulfate (Velban, Velsar, VLB), vincristine sulfate (Oncovin, Vincasar PFS, Vincrex), vinorelbine sulfate (Navelbine), etc.), chromatin function inhibitors (e.g., topotecan (Camptosar), irinotecan (Hycamtin), etoposide (VP-16, VePesid, Toposar), teniposide (VM-26, Vumon), etc.), hormones and hormone inhibitors (e.g. diethylstilbestrol (Stilbesterol, Stilphostrol), estradiol, estrogen, esterified estrogens (Estratab, Menest), estramustine (Emcyt), tamoxifen (Nolvadex), toremifene (Fareston) anastrozole (Arimidex), letrozole (Femara), 17-OH-progesterone, medroxyprogesterone, megestrol acetate (Megace), goserelin (Zoladex), leuprolide (Leupron), testosteraone, methyltestosterone, fluoxmesterone (Android-F, Halotestin), flutamide (Eulexin), bicalutamide (Casodex), nilutamide (Nilandron), etc.) INHIBITORS OF SYNTHESIS (e.g., amino-glutethimide (Cytadren), ketoconazole (Nizoral), etc.), immunomodulators (e.g., rituximab (Rituxan), trastuzumab (Herceptin), denileukin diftitox (Ontak), levamisole (Ergamisol), bacillus Calmette-Guerin, BCG (TheraCys, TICE BCG), interferon alpha-2a, alpha 2b (Roferon-A, Intron A), interleukin-2, aldesleukin (ProLeukin), etc.) and other agents such as 1-asparaginase (Elspar, Kidrolase), pegaspargase (Oncaspar), hydroxyurea (Hydrea, Doxia), leucovorin (Wellcovorin), mitotane (Lysodren), porfimer (Photofrin), tretinoin (Vesanoid), and the like.

### III. Pharmaceutical Compositions.

In order to carry out the methods of the invention, one or more active agents (chimeric moieties) of this invention are administered, e.g. to an individual diagnosed as having a cancer. The active agent(s) can be administered in the "native" form or, if desired, in the form of salts, esters, amides, prodrugs, derivatives, and the like, provided the salt, ester, amide, prodrug or derivative is suitable pharmacologically, i.e., effective in the present method. Salts, esters, amides, prodrugs and other derivatives of the active agents can be prepared using standard procedures known to those skilled in the art of synthetic organic chemistry and described, for example, by March (1992) *Advanced Organic Chemistry; Reactions, Mechanisms and Structure*, 4th Ed. N.Y. Wiley-Interscience.

For example, acid addition salts are prepared from the free base using conventional methodology, that typically involves reaction with a suitable acid. Generally, the base form of the drug is dissolved in a polar organic solvent such as methanol

or ethanol and the acid is added thereto. The resulting salt either precipitates or can be brought out of solution by addition of a less polar solvent. Suitable acids for preparing acid addition salts include both organic acids, e.g., acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, and the like, as well as inorganic acids, e.g., hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like. An acid addition salt may be reconverted to the free base by treatment with a suitable base. Particularly preferred acid addition salts of the active agents herein are halide salts, such as may be prepared using hydrochloric or hydrobromic acids. Conversely, preparation of basic salts of the active agents of this invention are prepared in a similar manner using a pharmaceutically acceptable base such as sodium hydroxide, potassium hydroxide, ammonium hydroxide, calcium hydroxide, trimethylamine, or the like. Particularly preferred basic salts include alkali metal salts, e.g., the sodium salt, and copper salts.

Preparation of esters typically involves functionalization of hydroxyl and/or carboxyl groups which may be present within the molecular structure of the drug. The esters are typically acyl-substituted derivatives of free alcohol groups, i.e., moieties that are derived from carboxylic acids of the formula RCOOH where R is alky, and preferably is lower alkyl. Esters can be reconverted to the free acids, if desired, by using conventional hydrogenolysis or hydrolysis procedures.

Amides and prodrugs can also be prepared using techniques known to those skilled in the art or described in the pertinent literature. For example, amides may be prepared from esters, using suitable amine reactants, or they may be prepared from an anhydride or an acid chloride by reaction with ammonia or a lower alkyl amine. Prodrugs are typically prepared by covalent attachment of a moiety that results in a compound that is therapeutically inactive until modified by an individual's metabolic system.

The active agents identified herein are useful for parenteral, topical, oral, nasal (or otherwise inhaled), rectal, or local administration, such as by aerosol or transdermally, for prophylactic and/or therapeutic treatment of one or more of the pathologies/indications described herein (e.g., atherosclerosis and/or symptoms thereof). The pharmaceutical compositions can be administered in a variety of unit dosage forms depending upon the method of administration. Suitable unit dosage forms, include, but are not limited to powders, tablets, pills, capsules, lozenges, suppositories, patches, nasal sprays, injectables, implantable sustained-release formulations, lipid complexes, etc.

The active agents of this invention are typically combined with a pharmaceutically acceptable carrier (excipient) to form a pharmacological composition. Pharmaceutically acceptable carriers can contain one or more physiologically acceptable compound(s) that act, for example, to stabilize the composition or to increase or decrease the absorption of the active agent(s). Physiologically acceptable compounds can include, for example, carbohydrates, such as glucose, sucrose, or dextrans, antioxidants, such as ascorbic acid or glutathione, chelating agents, low molecular weight proteins, protection and uptake enhancers such as lipids, compositions that reduce the clearance or hydrolysis of the active agents, or excipients or other stabilizers and/or buffers.

Other physiologically acceptable compounds include wetting agents, emulsifying agents, dispersing agents or preservatives that are particularly useful for preventing the growth

or action of microorganisms. Various preservatives are well known and include, for example, phenol and ascorbic acid. One skilled in the art would appreciate that the choice of pharmaceutically acceptable carrier(s), including a physiologically acceptable compound depends, for example, on the route of administration of the active agent(s) and on the particular physio-chemical characteristics of the active agent(s).

The excipients are preferably sterile and generally free of undesirable matter. These compositions may be sterilized by conventional, well-known sterilization techniques.

In therapeutic applications, the compositions of this invention are administered to a patient suffering e.g. from a cancer, or at risk of cancer (e.g. after surgical removal of a primary tumor) in an amount sufficient to prevent and/or cure and/or or at least partially prevent or arrest the disease and/or its complications. An amount adequate to accomplish this is defined as a "therapeutically effective dose." Amounts effective for this use will depend upon the severity of the disease and the general state of the patient's health. Single or multiple administrations of the compositions may be administered depending on the dosage and frequency as required and tolerated by the patient. In any event, the composition should provide a sufficient quantity of the active agents of the formulations of this invention to effectively treat (ameliorate one or more symptoms) the patient.

The concentration of active agent(s) can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration selected and the patient's needs. Concentrations, however, will typically be selected to provide dosages ranging from about 0.1 or 1 mg/kg/day to about 50 mg/kg/day and sometimes higher. Typical dosages range from about 3 mg/kg/day to about 3.5 mg/kg/day, preferably from about 3.5 mg/kg/day to about 7.2 mg/kg/day, more preferably from about 7.2 mg/kg/day to about 11.0 mg/kg/day, and most preferably from about 11.0 mg/kg/day to about 15.0 mg/kg/day. In certain preferred embodiments, dosages range from about 10 mg/kg/day to about 50 mg/kg/day. In certain embodiments, dosages range from about 20 mg to about 50 mg given orally twice daily. It will be appreciated that such dosages may be varied to optimize a therapeutic regimen in a particular subject or group of subjects.

In certain preferred embodiments, the active agents of this invention are administered orally (e.g. via a tablet) or as an injectable in accordance with standard methods well known to those of skill in the art. In other preferred embodiments, the peptides, may also be delivered through the skin using conventional transdermal drug delivery systems, i.e., transdermal "patches" wherein the active agent(s) are typically contained within a laminated structure that serves as a drug delivery device to be affixed to the skin. In such a structure, the drug composition is typically contained in a layer, or "reservoir," underlying an upper backing layer. It will be appreciated that the term "reservoir" in this context refers to a quantity of "active ingredient(s)" that is ultimately available for delivery to the surface of the skin. Thus, for example, the "reservoir" may include the active ingredient(s) in an adhesive on a backing layer of the patch, or in any of a variety of different matrix formulations known to those of skill in the art. The patch may contain a single reservoir, or it may contain multiple reservoirs.

In one embodiment, the reservoir comprises a polymeric matrix of a pharmaceutically acceptable contact adhesive material that serves to affix the system to the skin during drug delivery. Examples of suitable skin contact adhesive materials include, but are not limited to, polyethylenes, polysilox-

anes, polyisobutylenes, polyacrylates, polyurethanes, and the like. Alternatively, the drug-containing reservoir and skin contact adhesive are present as separate and distinct layers, with the adhesive underlying the reservoir which, in this case, 5 may be either a polymeric matrix as described above, or it may be a liquid or hydrogel reservoir, or may take some other form. The backing layer in these laminates, which serves as the upper surface of the device, preferably functions as a primary structural element of the "patch" and provides the 10 device with much of its flexibility. The material selected for the backing layer is preferably substantially impermeable to the active agent(s) and any other materials that are present.

In certain embodiments elevated serum half-life can be maintained by the use of sustained-release protein "packaging" systems. Such sustained release systems are well known to those of skill in the art. In one preferred embodiment, the ProLease™ biodegradable microsphere delivery system for proteins and peptides (see, e.g., Tracy (1998) *Biotechnol. Prog.* 14: 108; Johnson et al. (1996), *Nature Med.* 2: 795; 15 Herbert et al. (1998), *Pharmaceut. Res.* 15, 357) a dry powder composed of biodegradable polymeric microspheres containing the active agent in a polymer matrix that can be compounded as a dry formulation with or without other agents.

The ProLease™ microsphere fabrication process was specifically designed to achieve a high encapsulation efficiency while maintaining integrity of the active agent. The process consists of (i) preparation of freeze-dried drug particles from bulk by spray freeze-drying the drug solution with stabilizing excipients, (ii) preparation of a drug-polymer suspension followed by sonication or homogenization to reduce the drug particle size, (iii) production of frozen drug-polymer microspheres by atomization into liquid nitrogen, (iv) extraction of the polymer solvent with ethanol, and (v) filtration and vacuum drying to produce the final dry-powder product. The resulting powder contains the solid form of the active agents, which is homogeneously and rigidly dispersed within porous polymer particles. The polymer most commonly used in the process, poly(lactide-co-glycolide) (PLG), is both biocompatible and biodegradable.

Encapsulation can be achieved at low temperatures (e.g., -40° C.). During encapsulation, the protein is maintained in the solid state in the absence of water, thus minimizing water-induced conformational mobility of the protein, preventing protein degradation reactions that include water as a reactant, and avoiding organic-aqueous interfaces where proteins may undergo denaturation. A preferred process uses solvents in which most proteins are insoluble, thus yielding high encapsulation efficiencies (e.g., greater than 95%).

In another embodiment, one or more components of the solution can be provided as a "concentrate", e.g., in a storage container (e.g., in a premeasured volume) ready for dilution, or in a soluble capsule ready for addition to a volume of water.

The foregoing formulations and administration methods are intended to be illustrative and not limiting. It will be appreciated that, using the teaching provided herein, other suitable formulations and modes of administration can be readily devised.

#### IV. Kits.

In certain embodiments, this invention provides for kits for the treatment a primary cancer and/or in an adjunct therapy. Kits typically comprise a container containing a chimeric moiety of the present invention (e.g., anti-HER2/neu-IFN- $\alpha$ , anti-CD20-IFN- $\alpha$ , etc.). The chimeric moiety can be present in a pharmacologically acceptable excipient.

In addition the kits can optionally include instructional materials disclosing means of use of the chimeric moiety (e.g. to treat a cancer and/or as an adjunct therapeutic). The instruc-

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tional materials may also, optionally, teach preferred dosages, counter-indications, and the like.

The kits can also include additional components to facilitate the particular application for which the kit is designed. Thus, for example, and additionally comprise means for disinfecting a wound, for reducing pain, for attachment of a dressing, and the like.

While the instructional materials typically comprise written or printed materials they are not limited to such. Any medium capable of storing such instructions and communicating them to an end user is contemplated by this invention. Such media include, but are not limited to electronic storage media (e.g., magnetic discs, tapes, cartridges, chips), optical media (e.g., CD ROM), and the like. Such media may include addresses to internet sites that provide such instructional materials.

## EXAMPLES

The following examples are offered to illustrate, but not to limit the claimed invention.

### Example 1

#### Anti-Her2/Neu IgG3 and IFN-Alpha Fusion Protein Demonstrates Potent Apoptotic and Anti-Tumor Activities Against B Cell Lymphoma

In the present study, we constructed a fusion protein consisting of anti-HER2/neu-IgG3 with the variable region of C6MH3-B1 (20) and IFN- $\alpha$ , and investigated its effect on a murine B cell lymphoma, 38C13, expressing human HER2/neu (38C13/HER2). We chose to evaluate IFN- $\alpha$  targeting to tumor in this model given the responsiveness of this B cell lymphoma to IFN- $\alpha$  (21). Fusion of IFN- $\alpha$  to an Ab significantly increased its in vivo half-life. Anti-HER2/neu-IgG3-IFN- $\alpha$  was found to be efficient in inhibiting the growth in vivo of both small and established 38C13/HER2 tumors with no signs of systemic toxicity observed at effective doses. Anti-HER2/neu-IgG3-IFN- $\alpha$  inhibited the growth of and induced apoptosis in 38C13/HER2 cells. These results indicate that fusion of IFN- $\alpha$  to a tumor-specific Ab results in an agent effective for the treatment of B cell lymphoma.

#### Materials and Methods

##### Cell Lines and Culture Conditions

38C13 is a highly malignant murine B cell lymphoma derived from C3H/HeN mice. The construction and characterization of 38C13 expressing human HER2/neu (38C13/HER2) has been previously described (6). Both 38C13 and 38C13/HER2 were cultured in IMDM (Irvine Scientific) supplemented with 2 mM L-glutamine, 10 U/ml penicillin, 10 microg/ml streptomycin (GPS; Sigma-Aldrich) and 10% calf serum (Atlanta Biologicals). Murine myeloma P3X63Ag8.653 (American Type Culture Collection) and its derivatives expressing anti-HER2 IgG3-IFN- $\alpha$  or IgG3-IFN- $\alpha$  were grown in IMDM supplemented with 10% calf serum and GPS. L929 fibroblasts (American Type Culture Collection) were cultured in IMDM with 5% calf serum and GPS. The construction and characterization of CT26/HER2, a murine colon adenocarcinoma cell line overexpressing human HER2/neu, has been previously described (6). CT26/HER2 was cultured in IMDM with 5% calf serum and GPS.

##### Plasmid Construction

The H and L chain variable regions of C6MH3-B1, an anti-human HER2/neu scFv were inserted into the human  $\gamma$ 3 H chain (pAH4802) and  $\kappa$ Lchain (pAG4622) expression vectors, respectively (22), and used to produce chimeric IgG3 of

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this specificity. To construct the anti-human HER2/neu-IgG3 (C6MH3-B1)-IFN- $\alpha$  fusion protein, PCR was first used to introduce a BamH1 restriction enzyme site upstream and XbaI restriction enzyme site downstream of the mature murine IFN- $\alpha$  gene amplified by PCR from genomic DNA of BALB/c mice with the forward primer 5'-CGC GGA TCC TGT GAC CTG CCT CAG ACT C-3 (SEQ ID NO:58) and the reverse primer 5'-GCT CTA GAT CAT TTC TCT CTC AGT CTT C-3 (SEQ ID NO:59). The final PCR product was ligated into a TA vector. The resulting vector, after sequencing, was digested with BamH1 and XbaI to release the DNA fragment which was inserted into the vector pAH9612 containing the IgG3 constant region with the C6MH3-B1 H chain variable region and a GGGGSGGGSGGGGS (SEQ ID NO:60) peptide linker at the end of  $C_H$ 3. The final PCR product, pAH9616, contained anti-HER2/neu-IgG3 followed by a GGGGSGGGSGGGGS (SEQ ID NO:61) peptide linker and murine IFN- $\alpha$ .

#### Production and Purification of Recombinant Proteins

Plasmid encoding the IgG3 H chain with the C6MH3-B1 variable region fused to IFN- $\alpha$  was transfected into P3X63Ag8.653 cells expressing either L chain with the C6MH3-B1 variable region (23) to produce anti-HER2/neu-IgG3-IFN- $\alpha$  or nonspecific L chain (4D5; Genentech) (6) to produce IgG3-IFN- $\alpha$  by electroporation with a pulse of 960  $\mu$ Fd capacitance and 0.2 V. Transfectants producing anti-HER2/neu(C6MH3-B1)-IgG3, anti-HER2/neu(C6MH3-B1)-IgG3-IFN- $\alpha$ , or IgG3-IFN- $\alpha$  were selected and characterized as previously described (6). Anti-HER2/neu(C6MH3-B1)-IgG3 was purified from culture supernatants using protein G immobilized on Sepharose 4B fast flow (Sigma-Aldrich), and anti-HER2/neu(C6MH3-B1)-IgG3-IFN- $\alpha$  and IgG3-IFN- $\alpha$  were purified from culture supernatants using protein A immobilized on Sepharose 4B fast flow (Sigma-Aldrich). Purity and integrity were assessed by Coomassie blue staining of proteins separated by SDS-PAGE. The international reference standard for mouse IFN- $\alpha$  provided by the National Institutes of Health was used to determine IFN activity of the fusion proteins. rIFN- $\alpha$  was obtained from PBL Biomedical Laboratories.

#### FPLC Analysis of IgG3-IFN- $\alpha$ Fusion Protein

To determine whether the fusion protein exists as monomer and/or polymers in solution, 100  $\mu$ g of IgG3-IFN- $\alpha$  mixed with 400  $\mu$ g of OVA to provide an internal control was analyzed by gel filtration on a 30 $\times$ 1.5-cm Superose 6 column attached in a fast protein liquid chromatography (FPLC) using PBS and 0.5 ml/min flow rate. Gel filtration on the same column of IgA2m that exists predominantly as dimer Ab with a molecular mass of 350 kDa and a mixture of Miles IgG of molecular mass 150 kDa and OVA of molecular mass 45 kDa were used to provide molecular mass standards.

#### Flow Cytometry Analysis of HER2/Neu-Binding Activity

To detect the reactivity of various anti-HER2/neu fusion proteins with CT26/HER2 cells,  $1\times 10^6$  cells were incubated at 4° C. for 1 h with 10 pM of the fusion protein. For some experiments, the fusion proteins were preincubated with 900 U of heparin at 4° C. for 17 h before incubation with CT26/HER2 cells. Cells were then reacted with biotinylated rat anti-human IgG (BD Biosciences) diluted 1/100. The bound biotinylated Abs were detected with PE-labeled streptavidin (BD Biosciences) diluted 1/1500 and cells were analyzed by flow cytometry using a FACScan (BD Biosciences).

#### IFN- $\alpha$ Antiviral Activity

The L-929 fibroblast cell line sensitive to the vesicular stomatitis virus (VSV) infection was used to quantify the biological activity of IFN- $\alpha$ . L-929 cells were plated in a

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96-well tissue culture plate (Falcon; BD Biosciences) at a density of  $4 \times 10^4$  cells/well and incubated overnight at 37° C. in a 5% CO<sub>2</sub> atmosphere. Afterward, serial dilutions of different IFN- $\alpha$  fusion proteins or standard IFN- $\alpha$  (international reference standard for mouse IFN- $\alpha$ ; National Institutes of Health, Bethesda, Md.) were added and the plate was incubated at 37° C. for 24 h. Four thousand PFU of VSV was then added to each well and incubated at 37° C. for another 48 h. Surviving adherent cells were stained with 50  $\mu$ l of crystal violet (0.05% in 20% ethanol) for 10 min. The plates were washed with water and the remaining dye was solubilized by the addition of 100  $\mu$ l of 100% methanol. Plates were read using an ELISA reader at 595 nm.

#### Assay for the Antiproliferative Effect of Anti-HER2/Neu-IgG3-IFN- $\alpha$

In brief, 38C13 or 38C13/HER2 cells were plated in a 96-well tissue culture plate at a density of  $1.25 \times 10^4$  cells/well and serial dilutions of different fusion proteins were added. The plates were then incubated for 48 h at 37° C. in a 5% CO<sub>2</sub> atmosphere. Plates were developed by addition of 20  $\mu$ l of MTS solution (Promega) and analyzed at 490 nm using an ELISA reader. Inhibition of proliferation (percent) was calculated as:  $100 \times [(O_{Dexp} - O_{Dblank}) / (O_{Dmedium} - O_{Dblank})] \times 100$ .

#### Assay for Apoptosis

In brief,  $1 \times 10^6$  cells were treated with different fusion proteins for 72 h. The cells were then washed with ice-cold PBS. The annexin V/propidium iodide (PI) assay was conducted following procedures suggested by the manufacturer using the Vybrant Apoptosis Assay Kit 2 (Molecular Probes).

#### Proliferation of CFSE-Labeled 38C13/HER2 Tumor Cells

In brief,  $1 \times 10^6$  cells were incubated with 2.5  $\mu$ M CFSE (Molecular Probes) for 10 min at 37° C. Cells were then treated with 1 nM of different fusion proteins for 48 h and analyzed by flow cytometry following procedures suggested by the manufacturer using the CellTrace CFSE Cell Proliferation Kit (Molecular Probes).

#### Mice

Female C3H/HeN mice 6–8 wk of age obtained from Taconic Farms were used. Animals were housed in a facility using autoclaved polycarbonate cages containing wood-shaving bedding. The animals received food and water ad libitum. Artificial light was provided under a 12/12-h light/dark cycle. The temperature of the facility was 20° C. with 10–15 air exchanges per hour.

#### Half-Life

Murine rIFN- $\alpha$  (PBL Biomedical Laboratories), IgG3-IFN- $\alpha$ , and anti-HER2/neu-IgG3-IFN- $\alpha$  were iodinated to 10  $\mu$ Ci/ $\mu$ g with <sup>125</sup>I using Iodo-Beads (Pierce) according to the manufacturer's protocol. Mice were injected i.p. with 66  $\mu$ Ci of <sup>125</sup>I-labeled proteins. At various intervals after injection of <sup>125</sup>I-labeled rIFN- $\alpha$ , IgG3-IFN- $\alpha$ , or anti-HER2/neu-IgG3-IFN- $\alpha$ , residual radioactivity was measured using a mouse whole body counter (Wm. B. Johnson and Associates).

#### Tumor Challenge and Ab Therapy

C3H/HeN mice received 1000 38C13/HER2 tumor cells s.c. Treatment was given by i.p. injection either 1, 3, and 5 days or 12, 13, and 14 days after tumor challenge. Tumors were measured every other day, and the tumor volume (in cubic millimeters) was approximated using the following formula: [length (mm) × width (mm) × width (mm)]/2 (24). Animals were observed until the length of the s.c. tumor reached 15 mm or until any mouse was observed to be suffering or appeared to be moribund. Animals under these conditions were euthanized humanely according to institutional policy.

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#### Western Blot Analysis and Ab

In brief, 38C13/HER2 cells were treated with different fusion proteins for the indicated times, washed with ice-cold PBS, and lysed on ice for 10 min in lysis buffer (0.125% Nonidet P-40, 0.875% Brij 97, 10 mM Tris-HCl (pH 7.5), 2 mM EDTA, 0.15 M NaCl, 0.4 mM Na<sub>3</sub>VO<sub>4</sub>, 0.4 mM NaF, 1 mM PMSF, 2.5  $\mu$ M leupeptin, and 2.5  $\mu$ M aprotinin). Cell lysates were clarified at 10,000×g for 10 min at 4° C. Protein samples were then boiled in sample buffer before separation on 8% SDS-PAGE gels and transferred onto polyvinylidene fluoride microporous membranes (Millipore). After blocking with 3% BSA in 150 mM NaCl, 50 mM Tris-HCl (pH 7.6; TBS) for 1 h at room temperature, blots were probed with the indicated primary Abs overnight at 4° C. The blots were then washed three times at room temperature with 0.05% Tween 20 in TBS, incubated with the appropriate secondary Abs conjugated with HRP, and detected by a peroxidase-catalyzed ECL detection system (ECL; Pierce). Polyclonal rabbit antiphosphoSTAT1 was obtained from Cell Signaling Technology. Polyclonal HRP-conjugated donkey anti-rabbit IgG was obtained from Amersham Biosciences. Polyclonal rabbit anti-GAPDH was obtained from Abcam.

#### Statistical Analysis

Statistical analyses were performed using a two-tailed Student's t test for in vitro studies and log-rank (Mantel-Cox) analysis for animal survival curves.

#### Results

##### Production and Characterization of Anti-HER2/Neu-IgG3-IFN- $\alpha$

The construction and expression of anti-HER2/neu-IgG3 with the C6MH3-B1 (20) variable region has been described previously (23). The amino-terminal end of mature murine IFN- $\alpha$  was fused to the carboxyl-terminal end of anti-HER2/neu-IgG3 separated by a flexible [(Gly<sub>4</sub>)Ser]<sub>3</sub> (SEQ ID NO:31) linker (FIG. 2A). An identical fusion protein, IgG3-IFN- $\alpha$ , lacking HER2/neu specificity was constructed by replacing the C6MH3-B1 L chain with the 4D5 (rhuMab HER2, herceptin; Genentech) L chain. The proteins purified from culture supernatants using protein G were analyzed by SDS-PAGE under nonreducing and reducing conditions (FIG. 2B). In the absence of reducing agents, anti-HER2/neu-IgG3 (FIG. 2B, lane 1) migrates with a molecular mass of 170 kDa, whereas anti-HER2/neu-IgG3-IFN- $\alpha$  (FIG. 2B, lane 2) and IgG3-IFN- $\alpha$  (FIG. 2B, lane 3) are 210 kDa, the size expected for a complete IgG3 with two molecules of murine IFN- $\alpha$  attached (FIG. 2A). After treatment with the reducing agent, L chains migrating with a molecular mass of 25 kDa are seen for these proteins (FIG. 2B, lanes 4–6). However, the anti-HER2/neu-IgG3 has an H chain with a molecular mass of 60 kDa (FIG. 2B, lane 4), whereas IgG3-IFN- $\alpha$  (FIG. 2B, lane 5) and anti-HER2/neu-IgG3-IFN- $\alpha$  (FIG. 2B, lane 6) have an H chain with a molecular mass of 80 kDa as expected. The lower band in lane 1 (FIG. 2B) is bovine IgG which also bound to the protein G column; the bovine H and L chains are also seen in lane 4 (FIG. 2B) and to a lesser degree in lanes 5 and 6 (FIG. 2B). FPLC analysis showed that the IgG3-IFN- $\alpha$  fusion protein existed as a monomer in solution (data not shown).

##### Ag Binding and Antiviral Activity of Anti-HER2/Neu-IgG3-IFN- $\alpha$

Both anti-HER2/neu-IgG3 and anti-HER2/neu-IgG3-IFN- $\alpha$  bound CT26/HER2 cells, which express high levels of human HER2/neu, while IgG3-IFN- $\alpha$  bound CT26/HER2 weakly (FIG. 2C). Many cytokines including IL-1, IL-2, IL-6 (25) and IFN- $\alpha$  (26) have been shown to interact with heparin. To determine whether the weak interaction between IgG3-IFN- $\alpha$  and CT26/HER2 is due to the heparin binding, pro-

teins were incubated with heparin before the addition to CT26/HER2. Heparin inhibited the binding of IgG3-IFN- $\alpha$  to CT26/HER2 cells but did not inhibit the binding of anti-HER2/neu-IgG3 and anti-HER2/neu-IgG3-IFN- $\alpha$  (FIG. 2C).

These results demonstrated that anti-HER2/neu-IgG3-IFN- $\alpha$  retained its ability to bind Ag and IgG3-IFN- $\alpha$  does not recognize HER2/neu. The L-929 fibroblast cell line sensitive to VSV infection was used to quantify the IFN- $\alpha$  biological activity of the fusion proteins in comparison to an IFN- $\alpha$  standard. Both anti-HER2/neu-IgG3-IFN- $\alpha$  and IgG3-IFN- $\alpha$  exhibited ~2400 U of IFN- $\alpha$  activity/ $\mu$ g activity against VSV-induced cytotoxicity in L-929 cells, while anti-HER2/neu-IgG3 exhibited no anti-viral activity (FIG. 2D).

#### In Vivo Antitumor Activity of Fusion Proteins

To determine the in vivo antitumor activity of anti-HER2/neu-IgG3-IFN- $\alpha$ , syngeneic mice were inoculated s.c. with  $1 \times 10^3$  38C13/HER2 tumor cells and treated on days 1, 3, and 5 after tumor challenge by i.p. administration of different doses of protein (FIG. 3A-3B). Mice treated with 2.5  $\mu$ g of IgG3-IFN- $\alpha$  showed some regression of tumor growth, with one (13%) of eight mice alive after 50 days (FIG. 3A). However, in vivo targeting of IFN- $\alpha$  to tumors using a tumor-specific Ab dramatically improved its antitumor effect. All mice treated with 2.5  $\mu$ g (FIG. 3A) of anti-HER2/neu-IgG3-IFN- $\alpha$  remained tumor free 50 days after tumor challenge ( $p=0.0048$  compared with PBS control), and none of the treated mice showed evidence of toxicity. Thus, targeting of IFN- $\alpha$  to the tumor cell surface resulted in significant antitumor activity compared with IFN- $\alpha$  linked to a nonspecific Ab ( $p=0.007$ ). Targeted anti-HER2/neu-IgG3-IFN- $\alpha$  continued to show potent antitumor activity when a lower dose was used. Seven (88%) of eight mice treated with 1  $\mu$ g (FIG. 3B) of anti-HER2/neu-IgG3-IFN- $\alpha$  remained tumor free after 50 days. In marked contrast, at this lower dose mice treated with IgG3-IFN- $\alpha$  showed tumor growth similar to mice treated with PBS ( $p=0.183$ ) and only one (13%) of eight mice survived. When the treatment was increased to three doses of 5  $\mu$ g, both anti-HER2/neu-IgG3-IFN- $\alpha$  and IgG3-IFN- $\alpha$  were effective in preventing tumor growth (data not shown) undoubtedly reflecting the fact that 38C13 cells are sensitive to IFN- $\alpha$  treatment (21, 27, 28). Tumor growth in mice treated with 5  $\mu$ g of anti-HER2/neu-IgG3 Ab was the same as the PBS control, suggesting that Ab alone has no antitumor effect in vivo (data not shown). These results indicated that targeting of IFN- $\alpha$  to the tumor cells by a tumor-specific Ab can dramatically potentiate its effectiveness which was most clearly seen when low doses were administered. Importantly, this antitumor activity can be achieved without any evident toxicity.

#### IFN- $\alpha$ Fused to an Ab Results in Improved Antitumor Activity Compared with Free IFN- $\alpha$

As described above, we found that IFN- $\alpha$  fused to a non-tumorspecific Ab exhibited antitumor activity. To compare its antitumor activity with that of soluble rIFN- $\alpha$ , mice were inoculated s.c. with  $1 \times 10^3$  38C13/HER2 tumor cells and treated 1 and 3 days after tumor challenge by i.p. administration of 9600 U (4  $\mu$ g) of IgG3-IFN- $\alpha$  or 9600 U of rIFN- $\alpha$  (FIG. 4A). All mice treated with 9600 U of IgG3-IFN- $\alpha$  showed delayed tumor growth and 75% of the mice remained tumor free 50 days after tumor challenge ( $p=0.027$ ). In contrast, mice treated with the same number of units of rIFN- $\alpha$  were not statistically different from PBS controls in their tumor growth pattern.

IFN- $\alpha$  has a very short in vivo half-life (29). In previous study, fusion of Abs to cytokines has been shown to increase their halflife (6). The clearance of  $^{125}$ I-labeled rIFN- $\alpha$ , IgG3-IFN- $\alpha$ , or anti-HER2/neu-IgG3-IFN- $\alpha$  was examined in

C3H/HeN mice. Mice were injected i.p. with 66  $\mu$ Ci of  $^{125}$ I-labeled proteins and the residual radioactivity was measured using a mouse whole body counter. rIFN- $\alpha$  was cleared rapidly with 50% eliminated by ~2.5 h (FIG. 4B). In contrast, both anti-HER2/neu-IgG3-IFN- $\alpha$  and IgG3-IFN- $\alpha$  exhibited significantly increased in vivo half-life with ~8 h required for elimination of 50% of the injected radioactivity. This increased half-life may contribute to the antitumor efficacy of the IFN- $\alpha$  fusion proteins. Thus, fusion of an IgG3 Ab to IFN- $\alpha$  can significantly improve its in vivo antitumor activity. However, this antitumor activity can be further improved by targeting the IFN- $\alpha$  to the tumor, making it effective at lower doses.

#### Anti-HER2/Neu-IgG3-IFN- $\alpha$ Inhibited Proliferation of Tumor Cells In Vitro

IFN- $\alpha$  has multiple activities including activation of the immune response and direct cytotoxicity against tumors. To investigate potential mechanisms of the antitumor effects seen using either anti-HER2/neu-IgG3-IFN- $\alpha$  or IgG3-IFN- $\alpha$ , the eight mice remaining tumor free (see FIG. 3A) were challenged with  $1 \times 10^3$  38C13/HER2 tumor cells. Surprisingly, all mice resembled untreated mice and quickly developed bulky tumors (data not shown). These results imply that under these experimental conditions of low tumor burden the IFN- $\alpha$  fusion proteins did not initiate a protective adaptive immune response, but instead the potent antitumor activity of the IFN- $\alpha$  fusion proteins is mediated either by the innate immune system or by a direct cytotoxic effect on tumor cells.

To determine whether IFN- $\alpha$  fusion proteins are directly cytotoxic to tumor cells, the 38C13/HER2 or parental 38C13 tumor cells were incubated with different proteins for 48 h and cell proliferation measured using the MTS assay. Treatment with anti-HER2/neu-IgG3 did not significantly inhibit the proliferation of either 38C13/HER2 or parental 38C13 tumor cells (FIGS. 5A and 5B). Although both anti-HER2/neu-IgG3-IFN- $\alpha$  and IgG3-IFN- $\alpha$  inhibited the proliferation of 38C13/HER2 tumor cells, anti-HER2/neu-IgG3-IFN- $\alpha$  was more effective than IgG3-IFN- $\alpha$  with IPSO values of 10 and 100 pM for anti-HER2/neu-IgG3-IFN- $\alpha$  and IgG3-IFN- $\alpha$ , respectively (FIG. 5A). In contrast, anti-HER2/neu-IgG3-IFN- $\alpha$  and IgG3-IFN- $\alpha$  exhibited similar antiproliferative activity against parental 38C13 tumor cells. These results provided evidence that IFN- $\alpha$  fusion proteins can directly inhibit the proliferation of the B cell lymphoma 38C13, and targeting IFN- $\alpha$  to tumor cells potentiated this effect.

#### Anti-HER2/Neu-IgG3-IFN- $\alpha$ Induced Apoptosis in Tumor Cells In Vitro

IFN- $\alpha$  signaling can induce apoptosis in some tumor cell lines. To determine whether apoptosis contributed to the antiproliferative effect we observed, 38C13/HER2 cells treated with different proteins were assayed for the translocation of phosphatidylserine from the inner to the outer leaflet of the plasma membrane using the annexin V-affinity assay (30). Dead cells were stained by PI, which enters cells with a disrupted plasma membrane and binds to DNA. Compared with the PBS control, there was no increase in the number of dead cells (annexin V/PI bright, 2%) or early apoptotic cells (annexin V bright, 3%) following treatment with anti-HER2/neu-IgG3 (FIG. 5C). In contrast, when cells were treated with IgG3-IFN- $\alpha$ , there was a significant increase in the number of dead cells (21%) and early apoptotic cells (6%). Treatment with anti-HER2/neu-IgG3-IFN- $\alpha$  resulted in a further increase in both the number of dead cells (33%) and early apoptotic cells (16%). These results indicated that IFN- $\alpha$  can induce apoptosis in 38C13/HER2 tumor cells, and that targeting IFN- $\alpha$  to tumor cells can markedly increase this effect.

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In addition to inducing apoptosis, IFN- $\alpha$  can directly inhibit the proliferation of tumor cells (31). To determine whether both inhibition of proliferation and apoptosis were taking place in treated tumor cells, CFSE-labeled 38C13/HER2 cells were treated with different proteins for 48 h, the live cells were gated, and the level of CFSE was determined by flow cytometry. The CFSE signal in anti-HER2/neu-IgG3-treated cells (FIG. 5D, thin line) overlapped with the PBS-treated cells and was significantly less than that of cells fixed immediately after CFSE labeling (FIG. 5D, dotted line), indicating that anti-HER2/neu-IgG3 did not inhibit the proliferation of the 38C13/HER2. In contrast, IgG3-IFN- $\alpha$  significantly inhibited the proliferation of the surviving 38C13/HER2 cells (FIG. 5D, thick line), and targeting IFN- $\alpha$  to 38C13/HER2 cells by anti-HER2/neu-IgG3-IFN- $\alpha$  potentiated this effect (FIG. 5D, black area). These results indicated that although anti-HER2/neu-IgG3-IFN- $\alpha$  treatment did not result in complete cell death by 48 h, the surviving cells had a reduced ability to proliferate.

#### IFN- $\alpha$ Fusion Proteins Induce STAT1 Activation in Tumor Cells

Although engagement of the IFN- $\alpha$  receptor can initiate activation of multiple STAT proteins, STAT1 plays an obligate role in mediating IFN- $\alpha$ -dependent signaling (32). To investigate whether IFN- $\alpha$  fusion proteins initiate IFN- $\alpha$  signaling in 38C13/HER2 and that targeting IFN- $\alpha$  to tumor cells augments this effect, the phosphorylation of STAT1 following treatment was examined. As shown in FIG. 6A-6C, both anti-HER2/neu-IgG3-IFN- $\alpha$  and IgG3-IFN- $\alpha$  initiated robust STAT1 phosphorylation in 38C13/HER2 with STAT1 phosphorylation increasing 8-fold by 10 min. However, the phosphorylation of STAT1 induced by anti-HER2/neu-IgG3-IFN- $\alpha$  persisted for a longer period of time and greater STAT1 phosphorylation was seen at 30, 60, and 90 min in cells treated with anti-HER2/neu-IgG3-IFN- $\alpha$ . These results indicated that IFN- $\alpha$  fusion proteins can induce IFN- $\alpha$  signaling in 38C13 lymphoma cells and targeting IFN- $\alpha$  to tumor cells augments this effect.

#### Anti-HER2/Neu-IgG3-IFN- $\alpha$ Exhibited Potent Activity Against Established Tumors

Because anti-HER2/neu-IgG3-IFN- $\alpha$  exhibited potent cytotoxicity against 38C13/HER2 tumor cells, we investigated whether anti-HER2/neu-IgG3-IFN- $\alpha$  would be effective against established 38C13/HER2 tumors. Syngeneic mice were inoculated s.c. with  $1 \times 10^5$  38C13/HER2 tumor cells and i.p. treated with 5  $\mu$ g (FIG. 7) of the indicated proteins on days 12, 13, and 14 after tumor challenge. The average tumor size on day 12 is 100 mm<sup>3</sup> and treatment with PBS or 10  $\mu$ g of anti-HER2/neu-IgG3 (data not shown) did not inhibit tumor growth. Treatment with 5  $\mu$ g of IgG3-IFN- $\alpha$  showed some effect in inhibiting tumor growth; however, all mice developed bulky tumors and none of them survived 32 days after tumor challenge. In contrast all mice treated with 5  $\mu$ g of anti-HER2/neu-IgG3-IFN- $\alpha$  had delayed tumor growth, and three of eight mice had complete tumor regression and remained tumor free 50 days after tumor challenge (anti-HER2/neu-IgG3-IFN- $\alpha$  vs PBS,  $p=0.0001$ ; anti-HER2/neu-IgG3-IFN- $\alpha$  vs IgG3-IFN- $\alpha$ ,  $p=0.063$ ). Thus, both IgG3-IFN- $\alpha$  and anti-HER2/neu-IgG3-IFN- $\alpha$  showed anti-tumor activity but anti-HER2/neu-IgG3-IFN- $\alpha$  was more effective in delaying tumor growth and complete tumor remission was observed only in mice treated with anti-HER2/neu-IgG3-IFN- $\alpha$ . When the treatment dose was increased to 10  $\mu$ g of the fusion proteins, almost all mice treated with either anti-HER2/neu-IgG3-IFN- $\alpha$  or IgG3-IFN- $\alpha$  had complete tumor regression and remained tumor free after 50 days.

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The mice that remained tumor free following treatment with three doses of 10  $\mu$ g of fusion proteins were rechallenged with  $1 \times 10^5$  38C13/HER2 tumor cells on day 50. All mice remained tumor free (data not shown). These results suggest that an adaptive immune response with immunologic memory is initiated when larger, established tumors are treated with IFN- $\alpha$  fused to an Ab.

#### Discussion

Although rIFN- $\alpha$  has shown activity against B cell lymphoma and multiple myeloma, inconsistent efficacy and systemic toxicity have limited its usefulness (33). The present work demonstrates that fusing IFN- $\alpha$  to an Ab improves its efficacy against tumors with further improvement seen when IFN- $\alpha$  is targeted to tumor cells by a tumor-specific Ab. This antitumor efficacy is seen without any apparent toxicity. These studies suggest that fusion of IFN- $\alpha$  with tumor-specific Ab may yield an effective biologic agent for the treatment of B cell lymphoma.

To test the hypothesis that directing IFN- $\alpha$  to tumor sites with Ab would result in improved efficacy, we chose a well-characterized murine B cell lymphoma engineered to express a common TAA, HER2/neu, to which Abs are available. Anti-HER2/neu-IgG3-IFN- $\alpha$  appears to be more effective in the treatment of the 38C13 B cell lymphoma than previously described immunotherapeutics, although in the present study a foreign Ag introduced by gene transduction was the target. Treatment with three 1  $\mu$ g doses of anti-HER2/neu-IgG3-IFN- $\alpha$  beginning 1 day after tumor challenge appeared to be as effective in inhibiting tumor growth as treatment with 10  $\mu$ g of anti-Id IgG1-IL-2 fusion protein for 5 days beginning 1 day after tumor challenge (34). In addition, anti-HER2/neu-IgG3-IFN- $\alpha$  was effective against established tumors (FIG. 7) while anti-Id IgG1-IL-2 had little antitumor activity when treatment was begun either 3 or 7 days after tumor challenge (34). The ability to cure established tumors also suggests that Ab-targeted IFN- $\alpha$  is a more powerful therapeutic agent than GM-CSF (35), CTLA-4 (36), or CD40 ligand (37) fused to the Id Ag since none of these vaccine strategies was effective against established tumors. Therefore, targeting IFN- $\alpha$  to tumor cells could be a promising approach for treating B cell lymphoma.

Targeting IFN- $\alpha$  to tumor cells with a tumor-specific Ab increases the antitumor efficacy of IFN- $\alpha$ . Anti-HER2/neu-IgG3-IFN- $\alpha$  is more effective in inhibiting proliferation and inducing apoptosis (FIG. 5A-5D) in 38C13/HER2 than IgG3-IFN- $\alpha$  and treatment with either 2.5 or 1  $\mu$ g of anti-HER2/neu-IgG3-IFN- $\alpha$  was more effective in inhibiting growth of small tumors *in vivo* than the same doses of IgG3-IFN- $\alpha$  (FIGS. 3A and 3B). These results suggest that the tumor-specific Ab directs IFN- $\alpha$  to the tumor, thereby improving its therapeutic index with decreased systemic toxicity.

Remarkably, IgG3-IFN- $\alpha$  exhibits a more potent antitumor activity than rIFN- $\alpha$  (FIG. 4A). Although rIFN- $\alpha$  is effective in treatment of a variety of tumors (38-40), prolonged treatment with high doses is required to see effective antitumor activity in part because of the very short half-life of the cytokine. In this study, we demonstrated that fusion of an IgG3 Ab to IFN- $\alpha$  significantly increased its half-life (FIG. 4B), and this increased half-life may contribute to the increased *in vivo* antitumor activity of the fusion protein (FIG. 4A). In addition, the Fc region of the IgG3-IFN- $\alpha$  may help to target IFN- $\alpha$  to the Fc receptors present on B lymphoma cells and consequently increase the antitumor activity. Therefore, fusion of IFN- $\alpha$  to an IgG3 Ab may provide multiple advantages in improving the antitumor efficacy of IFN- $\alpha$ .

Although IFN- $\alpha$  has multiple activities, including activation of the immune response, it appears that direct cytotoxicity plays an important role in the potent antitumor activity of anti-HER2/neu-IgG3-IFN- $\alpha$ . Both IFN- $\alpha$  fusion proteins exhibited apoptotic and antiproliferative activities against 38C13/HER2 with tumor targeting significantly increasing these effects (FIG. 5A-5D). Although the IFN- $\alpha$  fusion proteins were very effective in treating small tumors (FIGS. 3A and 3B), none of the survivors developed an immune response that protected against second tumor challenge, suggesting that the direct cytotoxicity of the IFN- $\alpha$  fusion proteins was very effective in killing the tumor cells and that the adaptive immunity did not play a role when there was a small tumor burden. Because 38C13 is an extremely malignant B lymphoma cell line and mice injected with as few as 200 cells can develop bulky tumors within 20 days (36), the IFN- $\alpha$  fusion proteins must be very effective in killing most of the inoculated tumor cells to result in long-term survivors. Multiple mechanisms, including down-regulation of NF- $\kappa$ B (41), induction of apoptosis by activating caspase-3 (42), and up-regulation of both TRAIL and TRAIL receptors (43), have been shown to be involved in IFN- $\alpha$ -mediated cytotoxicity against tumor cells, and we would expect these mechanisms to contribute to the direct cytotoxicity against tumor cells seen with Ab-IFN- $\alpha$  fusion proteins. Consistent with this, we observed STAT1 activation following treatment of tumor cells with the fusion proteins (FIG. 6A-6C).

Although IFN- $\alpha$  fusion proteins failed to initiate a memory immune response when mice were treated beginning 1 day after tumor inoculation, IFN- $\alpha$  fusion proteins initiated an immune response that protected against second tumor challenge when mice were treated beginning 12 days after tumor inoculation. Therefore, IFN- $\alpha$  fusion proteins can activate protective adaptive immunity in the presence of a sizable tumor burden. Because IFN- $\alpha$  is capable of activating adaptive immunity via stimulation of DC differentiation and maturation (9), it is possible that the established tumors provide more TAAs for DC activation in the presence of IFN- $\alpha$ . In addition, the foreign Ag human HER2/neu may contribute to the antitumor immunity by increasing the immunogenicity of the tumor cells in this model.

CD20, an Ag expressed by B cells, is expressed in most B cell lymphomas (44), and anti-CD20 (rituximab, Genentech) is one of the most successful cancer therapeutics, having tremendous efficacy against lymphoma with little toxicity (45). Although anti-HER2/neu IgG3-IFN- $\alpha$  is very effective against 38C13/HER2, HER2/neu is not normally expressed in lymphoma cells and therefore, it probably has limited therapeutic application in the treatment of lymphoma but should be effective in the treatments of cancers that express HER2/neu. In contrast, fusion of IFN- $\alpha$  to anti-CD20 is expected to yield a fusion protein effective against lymphoma with even greater antitumor activity by combining the anti-lymphoma activity of anti-CD20 and the potent immunostimulatory and cytotoxic activity of IFN- $\alpha$  in one protein. Additionally, IFN- $\alpha$  may further up-regulate CD20 expression as was seen in patients with B cell lymphoma following IFN- $\alpha$  treatment (46). We are currently studying the effects of anti-CD20-IFN- $\alpha$  fusion proteins in murine models of B cell lymphoma.

In summary, we have constructed and characterized a novel fusion protein in which IFN- $\alpha$  was linked to an antibody recognizing a TAA. Our results indicate that fusion of IFN- $\alpha$  to a tumor-specific antibody can dramatically increase the efficacy of IFN- $\alpha$  with antitumor activity observed without any apparent toxicity. Remarkably, the Ab-IFN- $\alpha$  fusion protein was effective against established tumors. Therefore, IFN

(e.g., IFN- $\alpha$ ) fused to a tumor-specific antibody shows promise for the treatment of B cell lymphoma.

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## Example 2

Anti-CD20-IFN $\alpha$  Fusion Proteins

## Introduction

Our initial studies had indicated that a fusion protein with anti-HER2/neu joined to IFN- $\alpha$  was an effective therapeutic for the treatment of HER2/neu expressing lymphoma. We sought to extend these studies to show that fusion of IFN- $\alpha$  with anti-CD20 would be an effective therapeutic for treating CD20 expressing lymphomas. CD20 is present on virtually all lymphomas. However, it should be noted that HER2/neu is expressed on many cancers and it would be expected that the anti-HER2/neu fusion protein would be effective in treating these. In the anti-CD20 fusion protein, we would expect the IFN- $\alpha$  in the fusion protein to both exert a direct cytotoxic effect against the tumor cells and to help elicit an anti-tumor immune response.

## Produce Recombinant Antibodies Specific for CD20.

The variable regions for anti-CD20 (Rituximab) were amplified and cloned into expression vectors for the production of chimeric antibodies with human kappa light chains and gamma 3 heavy chains. Protein was produced and its ability to recognize CD20 examined using flow-cytometry and the human B-cell line Daudi. As shown in FIG. 8, the recombinant protein binds as well as Rituximab a recombinant IgG1.

## Produce Antibody Fusion Proteins with Human Interferon Joined to Antibodies Specific for CD20

## a. Design of Fusion Protein

In our initial attempt to make a fusion protein we joined IFN- $\alpha$  to the carboxy-terminus of the human IgG3 gene using a flexible glycine-serine linker consisting of (Gly<sub>4</sub>Ser)<sub>3</sub> (SEQ ID NO:31). The heavy chain is shown diagrammatically in FIG. 9.

After verifying that the fusion protein vector had the correct nucleotide sequence, it was transfected with the chimeric anti-CD20 light chain into NS0 cells. Transfectants were screened by ELISA for the production of IgG. The clone giving the highest signal was expanded and following sub-cloning was grown in roller bottles. Supernatants were then passed through protein A Sepharose columns, and the bound proteins eluted and analyzed by SDS-PAGE both unreduced and following reduction (see, FIG. 10). Although the isolated protein was assembled into H<sub>2</sub>L<sub>2</sub> molecules, most of the isolated protein was smaller than expected. Following reduction, most of the heavy chains were smaller than expected and ran at the same position as a gamma-3 heavy chain lacking a fusion protein. It appeared that the interferon was being removed from the fusion protein by proteolysis. Western blot analysis using anti-Fc and anti-interferon, confirmed that both of the upper bands were heavy chains, but only the largest contained interferon.

Flexible linkers can be a target of proteolytic cleavage. Therefore, we shortened the linker to only one copy of Gly<sub>4</sub>Ser (SEQ ID NO:32). These vectors and vectors with the extended linker were transiently transfected along with the appropriate light chain into HEK293T-cells. Cells were radiolabeled by growth in <sup>35</sup>S-methionine, immunoglobulins precipitated with protein A and analyzed by SDS-PAGE (FIG. 11). Whereas cleavage of fusion proteins with extended

linkers is readily apparent, cleavage does not take place when the linker consists of only one Gly<sub>4</sub>Ser (SEQ ID NO:32). Therefore, the linker used to produce the fusion protein is important and can influence its stability.

## b. Recognition of CD20 by the Fusion Proteins

To determine if the fusion protein recognizes CD20, the human cell line Daudi which expresses CD20 was incubated with Rituxan, anti-DNS/IgG3-hu-IFN- $\alpha$  or anti-CD20/IgG3-hu-IFN- $\alpha$ . The anti-CD20/IgG3-hu-IFN- $\alpha$  bound better than Rituxan (FIG. 12). The anti-DNS/IgG3-hu-IFN- $\alpha$  fusion also showed some binding, although less than either CD20 specific protein. We hypothesize that the binding of the anti-DNS/IgG3-hu-IFN- $\alpha$  and the enhanced binding of anti-CD20/IgG3-hu-IFN- $\alpha$  compared to Rituxan is because the hu-IFN- $\alpha$  moiety binds to IFN receptors expressed on the Daudi cells

The Timmerman laboratory has produced a transfectant of the murine lymphoma 38C13 that expresses human CD20. Both Rituxan and anti-CD20/IgG3-mu-IFN- $\alpha$  bound the transfectant. Anti-DNS/IgG3-mu-IFN- $\alpha$  showed no binding (FIG. 13).

## c. Anti-Viral Activity of the Fusion Proteins

To assess the anti-viral activity of the hu-IFN- $\alpha$  fusion proteins, HeLa cells were seeded at 2 $\times$ 10<sup>5</sup> cells/ml and treated with two-fold serial dilutions of fusion protein or Roferon (recombinant human interferon 2a) for 24 hrs. Cells were then infected with VSV (vesicular stomatitis virus) at a concentration of 4000 pfu/100  $\mu$ l. After 72 hrs, cells were stained with 0.1% crystal violet. Protection against viral infection was determined either by quantitating the cells surviving the infection by staining with 0.1% crystal violet and determining the amount of dye in each well using a spot densitometer or by counting the number of plaques. In both assays the fusion protein had significant IFN- $\alpha$  activity but was about 100-fold reduced in activity compared to Roferon.

## Growth Inhibition and Killing of Daudi Lymphoma Cells with the Fusion Proteins.

Two methods were used to assess the growth inhibition/killing of lymphoma cells expressing CD20 by the fusion proteins. It should be noted that for these experiments a human cell line, Daudi, that naturally expresses CD20 was used. In the first approach Daudi cells were incubated with various concentrations of IFN- $\alpha$ , antibody or fusion protein for 72 hrs and growth inhibition assessed using the CellTiter 96 AQueous cell proliferation assay (FIG. 14). Although showing less IFN- $\alpha$  activity in the anti-viral assay, anti-CD20/IgG3-hu-IFN- $\alpha$  and Roferon showed a similar ability to inhibit lymphoma growth suggesting that targeting the IFN- $\alpha$  enhances its cytotoxic effect. Anti-CD20/IgG3+ Roferon did not show enhanced activity compared to Roferon alone. Anti-DNS/IgG3-hIFN- $\alpha$ , Rituxan and anti-CD20/IgG3 only showed some growth inhibition at the highest concentration used. It should be noted that fusion protein was more active than Rituxan in preventing cell growth in this assay.

In the second approach, Daudi cells were incubated with various concentrations of IFN- $\alpha$ , antibody or fusion protein for 72 hrs and then stained with Annexin V and propidium iodide (PI) analyzed by FLOW cytometry. Shown in FIG. 15 are the results obtained when 10 pM of the various proteins was used. Cells in the early phases of apoptosis are Annexin V<sup>+</sup>PI<sup>-</sup>; late apoptotic and dead cells are Annexin V<sup>+</sup>PI<sup>+</sup>.

These experiments demonstrate several things. Rituxan and anti-CD20/IgG3 both induce little to no apoptosis, even at the highest concentrations tested. As would be expected, murine IFN- $\alpha$  is less effective against the human cell line than is human recombinant IFN- $\alpha$  (Roferon) and anti-DNS/IgG3-

mIFN α which would not target the tumor cells is approximately as effective as recombinant murine IFN-α. However, targeting murine IFN-α to tumor cells using anti-CD20/IgG3-mIFNα results in effective induction of cell death. Anti-CD20/IgG3-hIFNα is more effective than anti-DNS/IgG3-hIFN α, again demonstrating the contribution of cell targeting to cell killing. In this *in vitro* assay, Roferon and anti-CD20/IgG3-hIFNα exhibit similar activity causing cell death at concentrations as low as 1 pM (data not shown). However, it should be pointed out that *in vivo* CD20/IgG3-hIFNα will target and accumulate at the site of the tumor while Roferon will exhibit its activity throughout the body.

#### Growth Inhibition and Killing of 38C13-CD20 Lymphoma Cells with the Fusion Proteins

As briefly mentioned above, the laboratory of Dr. John Timmerman has developed a murine lymphoma, 38C13-CD20, that expresses human CD20 and will grow in syngenic C3H/HeJ mice. The availability of this cell line makes it possible to examine the *in vivo* efficacy of our fusion proteins. 38C13-CD20 cells were incubated for 48 hours with various antibodies and fusion proteins. Killing and apoptosis were then determined by staining cells with Annexin V and PI and examining them using FLOW cytometry. When proteins were used at a concentration of 100 pM (data not shown) both recombinant mIFN-α and anti-CD20-IgG3-mIFN-α were very effective in causing apoptosis, with anti-CD20-IgG3-mIFN-α somewhat more effective than recombinant mIFN-α. Some apoptosis was induced by treating 38C13-CD20 cells with anti-DNS-IgG3-mIFN-α or Rituxan. Treatment with anti-CD20/IgG3 at this concentration had no effect on cell viability. When the treatment concentration was lowered to 10 pM (FIG. 16), recombinant mIFN-α and anti-CD20/IgG3-mIFN-α continued to be effective in causing apoptosis, with anti-CD20/IgG3-mIFN-α more effective than recombinant mIFN-α. Only a small amount of apoptosis was seen following treatment with anti-DNS-IgG3-mIFN-α indicating that targeting of IFN-α using anti-CD20-IgG3-mIFN-α resulted in a more effective therapeutic agent. At this concentration Rituxan caused little apoptosis, indicating the superiority of the anti-CD20-IgG3/mIFN-α fusion protein over the unfused anti-CD20 antibody. Again, treatment with anti-CD20/IgG3 had no effect on cell viability. At a treatment dose of 1 pM, only anti-CD20-IgG3-mIFN-α induced apoptosis in 38C13-CD20 (data not shown). At a dose of 0.1 pM, none of the treatments induced apoptosis (data not shown).

As an alternative approach, 38C13-CD20 cells were treated with the various proteins at different concentrations and inhibition of growth monitored using the MTS assay (FIG. 17). Anti-CD20/IgG3-mIFN-α was most effective in inhibiting cell growth, followed by recombinant mIFN-α. Some growth inhibition was observed with anti-DNS/IgG3-mIFN-α. Anti-CD20/IgG3 and Rituxan had little effect on cell growth. Thus, the results obtained in this assay mirrored what was observed when apoptosis was monitored.

#### Production and Characterization of Additional IgG-IFNα Fusion Proteins

##### a. Anti-CD20-IgG1-mIFNα and Anti-CD20-IgG1-hIFNα

The initial proteins were made with IFN-α fused to a human IgG3 backbone. Rituxan is an IgG1. To determine if the immunoglobulin backbone influenced the properties of the fusion proteins, fusion proteins with m-IFN-α and hu-IFN-α fused to IgG1 have now been produced. They were of the expected molecular weight.

Anti-CD20/IgG1-mIFNα was evaluated for its ability to induce apoptosis of 38C13-CD20 (FIG. 18). The studies showed it to be effective, possibly even more effective than the IgG3 fusion protein.

Anti-CD20/IgG1-hIFNα was evaluated for its ability to induce apoptosis of Daudi cells. The studies showed it exhibit activity similar to anti-CD20/IgG3-hIFNα (FIG. 19)

The fusion proteins were evaluated for their ability to inhibit the growth of Daudi cells as shown in FIG. 20. IgG1 fusions with both murine and human IFNα resembled the IgG3 fusions in their ability to inhibit the growth of Daudi cells.

##### b. Fusion Proteins with IFN-α Joined to the IgG Backbone with an Alpha Helical Linker

Fusion proteins were produced in which the GlySer linker was replaced with linker with the sequence A(EAAAK)<sub>2</sub>A (SEQ ID NO:33). This sequence is proposed to fold as an alpha helix.

Protein was produced by transient expression in 293T cells and evaluated by SDS-PAGE. The protein assembled and was of the expected molecular weight. No cleavage of the linker was observed.

The fusion protein, anti-CD20-IgG3-hIFNα (α-helical linker) when used at the same concentration as the fusion protein with the Gly<sub>4</sub>Ser (SEQ ID NO:32) linker, was found to effectively induce apoptosis of Daudi cells (FIG. 21).

#### In Vivo Treatment of Tumors

The 38C13 lymphoma that had been transduced by the Timmerman laboratory to express human CD20 was used for these studies. 38C13 is an aggressive lymphoma that grows in syngenic C3H/HeJ mice. The transductant, 38C13-CD20, exhibits the same growth characteristic. Thus it is possible to investigate fusion protein mediated protection in immune competent animals.

##### a. Treatment of Early Tumors

Mice (groups of 4) were injected subcutaneously with 5000 38C13-CD20 cells on day zero. On days 1, 2 and 3 they were treated intravenously with hepes buffered saline solution (HBSS) or 0.4 µg, 2 µg, or 10 µg of anti-CD20-m-IFN-α and tumor growth monitored. By day 20 all of the animals treated with HBSS had large tumors and had to be sacrificed. In contrast, no tumor growth was seen in animals treated with 10 µg of the fusion protein; after day 20 tumors began to grow in 3 of the four animals treated with 0.4 µg of the fusion protein and 1 of the mice treated with 2 µg. The results showed that the anti-CD20/IFN-α fusion proteins are very effective in inhibiting *in vivo* tumor growth and in increasing survival (see, e.g., FIG. 22).

##### b. The Anti-CD20-mIFNα Fusion Protein is More Effective than Either Rituximab or Anti-CD20/IgG3 in Treating Moderate Sized Tumors

C3H/HeJ mice were inoculated with 5000 38C13-CD20 cells on day 0. On days 5, 6 and 7 they were treated with HBSS or 10 µg of anti-CD20-IgG1 (produced in 293T cells), anti-CD20-IgG3, Rituximab or anti-CD20-IgG3-mIFNα. They were monitored for tumor growth and survival (see, e.g., FIG. 23). Anti-CD20/IgG3-mIFNα was much more effective than Rituximab, anti-CD20/IgG3 or anti-CD20/IgG1 in preventing the growth of moderate sized tumors.

#### The Tumor Targeting Ability of the Fusion Protein Significantly Enhances its Efficacy in Vivo.

C3H/H3J mice were inoculated with 5000 38C13-CD20 cells on day 0 and treated on days 5, 6 and 7 with 10 µg of anti-CD20-IgG3, 10 µg of anti-CD20-IgG3+mIFN-α (dose chosen to be same moles as in fusion protein), anti-DNS-IgG3-IFNα, or anti-CD20-IgG3-mIFNα and followed for tumor growth and survival (see, e.g., FIG. 24). Anti-CD20-IgG3-IFNα significantly delayed tumor growth and promoted survival indicating that targeting the IFNα to the tumor using the antibody combining site makes it a more effective therapeutic than either a fusion protein that does not target the fused IFNα (anti-DNS-IgG3-IFNα) or the injection of anti-CD20 along with IFNα that is not covalently associated (anti-CD20-IgG3+mIFN-α).

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Fusion Protein Treatment is Effective Against Established Tumors

Groups of eight C3H/HeJ mice were inoculated with 5000 38C13-CD20 cells and treated on days 8, 9 and 10 with 100 µg of anti-CD20-mIFN $\alpha$  or HBSS. Mice were monitored for tumor growth (see FIG. 25) and survival (see, FIG. 26). Mice inoculated with anti-CD20-mIFN $\alpha$  shows improved survival (FIG. 26).

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It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

## SEQUENCE LISTING

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  35         40              45

Thr Ser Tyr Trp Ile Ala Trp Val Arg Gln Met Pro Gly Lys Gly Leu
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Glu Tyr Met Gly Leu Ile Tyr Pro Gly Asp Ser Asp Thr Lys Tyr Ser
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Pro Ser Phe Gln Gly Gln Val Thr Ile Ser Val Asp Lys Ser Val Ser
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 195        200             205

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
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Gly Thr Gln Thr Tyr Thr Cys Asn Val Asn His Lys Pro Ser Asn Thr
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 355 360 365  
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 Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser  
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 Pro Glu Asn Asn Tyr Asn Thr Thr Pro Pro Met Leu Asp Ser Asp Gly  
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## US 9,139,634 B2

**49****50**

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Leu Leu Ile Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg
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Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser
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Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
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Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
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Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
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Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
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## US 9,139,634 B2

**51****52**

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Val Gln Ser Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys		
20	25	30

Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe		
35	40	45

Thr Ser Tyr Asn Met His Trp Val Lys Gln Thr Pro Gly Arg Gly Leu

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50	55	60
Glu Trp Ile Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn		
65	70	75
80		
Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser		
85	90	95
Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val		
100	105	110
Tyr Tyr Cys Ala Arg Ser Thr Tyr Tyr Gly Gly Asp Trp Tyr Phe Asn		
115	120	125
Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ala Ala Ser Thr Lys		
130	135	140
Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Gly		
145	150	155
160		
Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro		
165	170	175
Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr		
180	185	190
Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val		
195	200	205
Val Thr Val Pro Ser Ser Leu Gly Thr Gln Thr Tyr Thr Cys Asn		
210	215	220
Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Gly Glu		
225	230	235
240		
Arg Pro Ala Gln Gly Gly Arg Val Ser Ala Gly Ser Gln Ala Gln Pro		
245	250	255
Ser Cys Leu Asp Ala Ser Arg Leu Cys Ser Pro Ser Pro Gly His Gln		
260	265	270
Gly Arg Pro Arg Leu Thr Pro His Pro Glu Ala Ser Ala Arg Pro Thr		
275	280	285
His Ala Gln Gly Glu Gly Leu Leu Ala Phe Ser Thr Arg Leu Arg Ala		
290	295	300
Gly Thr Gly Trp Met Pro Leu Pro Gln Ala Leu His Thr Gln Gly Gln		
305	310	315
320		
Val Leu Arg Ser Glu Leu Pro Arg Ala Ile Ser Arg Arg Thr Leu Pro		
325	330	335
Leu Thr Glu Leu Lys Thr Pro Leu Gly Asp Thr Thr His Thr Cys Pro		
340	345	350
Arg Cys Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg		
355	360	365
Cys Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys		
370	375	380
Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro		
385	390	395
400		
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser		
405	410	415
His Glu Asp Pro Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu		
420	425	430
Val His Asn Ala Lys Thr Lys Leu Arg Glu Glu Gln Tyr Asn Ser Thr		
435	440	445
Phe Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn		
450	455	460
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro		
465	470	475
480		

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Ile Glu Lys Thr Ile Ser Lys Ala Lys Met Thr Lys Asn Gln Val Ser  
485 490 495

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu  
500 505 510

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Asn Thr Thr Pro Pro  
515 520 525

Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val  
530 535 540

Asp Lys Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met  
545 550 555 560

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser  
565 570 575

Pro Gly Lys Ser Gly Gly Gly Ser Cys Asp Leu Pro Gln Thr His  
580 585 590

Asn Leu Arg Asn Lys Arg Ala Leu Thr Leu Leu Val Gln Met Arg Arg  
595 600 605

Leu Ser Pro Leu Ser Cys Leu Lys Asp Arg Lys Asp Phe Gly Phe Pro  
610 615 620

Gln Glu Lys Val Asp Ala Gln Gln Ile Lys Lys Ala Gln Ala Ile Pro  
625 630 635 640

Val Leu Ser Glu Leu Thr Gln Gln Ile Leu Asn Ile Phe Thr Ser Lys  
645 650 655

Asp Ser Ser Ala Ala Trp Asn Ala Thr Leu Leu Asp Ser Phe Cys Asn  
660 665 670

Asp Leu His Gln Gln Leu Asn Asp Leu Gln Gly Cys Leu Met Gln Gln  
675 680 685

Val Gly Val Gln Glu Phe Pro Leu Thr Gln Glu Asp Ala Leu Leu Ala  
690 695 700

Val Arg Lys Tyr Phe His Arg Ile Thr Val Tyr Leu Arg Glu Lys Lys  
705 710 715 720

His Ser Pro Cys Ala Trp Glu Val Val Arg Ala Glu Val Trp Arg Ala  
725 730 735

Leu Ser Ser Ser Ala Asn Val Leu Gly Arg Leu Arg Glu Glu Lys  
740 745 750

<210> SEQ ID NO 7  
<211> LENGTH: 2279  
<212> TYPE: DNA  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Nucleic acid encoding fusion protein.

<400> SEQUENCE: 7

atgtacttgg	gactgaactg	tgtaatcata	gttttctct	taaaaggtgt	ccagagtcag	60
gtacaactgc	agcagcctgg	ggctgagctg	gtgaaggctg	gggcctcagt	gaagatgtcc	120
tgcaaggcct	ctggctacac	atttaccagt	tacaatatgc	actgggtaaa	acagacacct	180
ggtcggggcc	tggaatggat	tggagctatt	tatcccgaa	atggtgatac	ttcctacaat	240
cagaagttca	aaggcaaggc	cacattgact	gcagacaaat	cctccagcac	agcctacatg	300
cagctcagca	gcctgacatc	tgaggactct	gcggctatt	actgtgcaag	atcgacttac	360
tacggcgggt	actggtaactt	caatgtctgg	ggcgccaggaa	ccacggtcac	cgtctctgca	420
gctagcacca	agggccatc	ggtcttcccc	ctggcgccct	gctccaggag	cacctctggg	480
ggcacacagcg	ccctgggctg	cctggtcaag	gactacttcc	ccgaaccggt	gacggtgtcg	540

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tggaaactcgac ggcgcctgac cagcggcggtg cacacccccc cggctgtccct acagtctca  
ggactctact ccctcagcag cgtggtgacc gtgcctcca gcagcttggg caccaggacc  
tacacctgcac acgtgaatca caagccccgc aacaccaagg tggacaagag agttggtag  
aggccagcgc agggagggag ggtgtctgt ggaagccagg ctcagccctc ctgectggac  
gcataccggc tgcgtcagttcc cagccccagg caccaaggca ggccccgtct gactcctcac  
ccggaggccct ctgcggccccc cactcatgt cagggaggg gtccttgcc ttttccacc  
aggctccggc caggcacagg ctggatgccc ctaccccaagg cccttcacac acaggggcag  
gtgctgcgtc cagagctgcc aagagccata tccaggagga ccctgcctt gaccgagtc  
aaaacccac ttggtgacac aactcacaca tgcccacgg gcccagagcc caaatcttgt  
gacacacccctc cccctgtcccc aaggtgcccc gagcccaaat ctgtgacac acctccccccg  
tgcccaaggt gcccagagcc caaatcttgt gacacacccctc cccctgtcccc aaggtgcccc  
tgattttccggc gacccctgag gtcacgtcg tgggtgtggc cgtgagccac gaagccccgg  
aggtcccgattt caagtggtagt gttggacggcg tggaggtgca taatgccaag acaaagctgc  
gggaggagca gtacaacagc acgttccgtg tggtagcgt cctcacccgtc ctgcaccagg  
actggctgaa cggcaaggag tacaagtgc aagttctccaa caaagccctc ccagccccca  
tcgagaaaaac catctccaaa gccaaaaatga ccaagaacca ggtcagccctg acctgcctgg  
tcaaaggctt ctaccccaagc gacatcgccg tggagttggg gagcaatggg cagccggaga  
acaactacaa caccacgcct cccatgtgg actccgacgg ctcccttctc ctctacagca  
agctcacccgt ggtacaagagc aggtggcgc aggggaacat ctctcatgc tccgtatgc  
atgaggctct gcacaaccac tacacgcaga agagcccttc cctgttcccg ggtaaagcag  
aggccgcgc taaagaggcc gcagccaaag cgggatctgt tgacctgcct cagactcata  
acctcgaggaa caagagagcc ttgacactcc tggtagccaaat gaggagactc tccctctct  
cctgcctgaa ggacagggaaag gactttggat tcccgccaggaa gaggtggat gcccaggaga  
tcaagaaggc tcaaggccatc cctgtcctga gtgagctgac ccagcagatc ctgaaacatct  
tcacatcaa ggactcatct gctgttggaa atgcaaccct cctagactca ttctgtcaat  
acctccacca gcagctcaat gacctgcag gttgtctgt gacgcagggt ggggtgcagg  
aattttccctt gacccaggaa gatggccctgc tggctgttag gaaatacttc cacaggatca  
ctgtgtacccctt gagagagaag aaacacagcc cctgtgcctg ggaggtggc agagcagaag  
tctggagagc cctgttcccttcc tctgccaatg tgctggaaag actgagagaa gagaaatga

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<210> SEQ ID NO 8
<211> LENGTH: 759
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Fusion protein.
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<400> SEQUENCE: 8

Met	Tyr	Leu	Gly	Leu	Asn	Cys	Val	Ile	Ile	Val	Phe	Leu	Leu	Lys	Gly
1				5					10					15	

Val Gln Ser Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys  
                   20                 25                 30

Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe  
                   35                 40                 45

Thr Ser Tyr Asn Met His Trp Val Lys Gln Thr Pro Gly Arg Gly Leu

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50	55	60
Glu Trp Ile Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn		
65	70	75
80		
Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser		
85	90	95
Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val		
100	105	110
Tyr Tyr Cys Ala Arg Ser Thr Tyr Tyr Gly Gly Asp Trp Tyr Phe Asn		
115	120	125
Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ala Ala Ser Thr Lys		
130	135	140
Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Gly		
145	150	155
160		
Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro		
165	170	175
Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr		
180	185	190
Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val		
195	200	205
Val Thr Val Pro Ser Ser Leu Gly Thr Gln Thr Tyr Thr Cys Asn		
210	215	220
Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Gly Glu		
225	230	235
240		
Arg Pro Ala Gln Gly Gly Arg Val Ser Ala Gly Ser Gln Ala Gln Pro		
245	250	255
Ser Cys Leu Asp Ala Ser Arg Leu Cys Ser Pro Ser Pro Gly His Gln		
260	265	270
Gly Arg Pro Arg Leu Thr Pro His Pro Glu Ala Ser Ala Arg Pro Thr		
275	280	285
His Ala Gln Gly Glu Gly Leu Leu Ala Phe Ser Thr Arg Leu Arg Ala		
290	295	300
Gly Thr Gly Trp Met Pro Leu Pro Gln Ala Leu His Thr Gln Gly Gln		
305	310	315
320		
Val Leu Arg Ser Glu Leu Pro Arg Ala Ile Ser Arg Arg Thr Leu Pro		
325	330	335
Leu Thr Glu Leu Lys Thr Pro Leu Gly Asp Thr Thr His Thr Cys Pro		
340	345	350
Arg Cys Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg		
355	360	365
Cys Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys		
370	375	380
Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro		
385	390	395
400		
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser		
405	410	415
His Glu Asp Pro Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu		
420	425	430
Val His Asn Ala Lys Thr Lys Leu Arg Glu Glu Gln Tyr Asn Ser Thr		
435	440	445
Phe Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn		
450	455	460
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro		
465	470	475
480		

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Ile Glu Lys Thr Ile Ser Lys Ala Lys Met Thr Lys Asn Gln Val Ser  
485 490 495

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu  
500 505 510

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Asn Thr Thr Pro Pro  
515 520 525

Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val  
530 535 540

Asp Lys Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met  
545 550 555 560

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser  
565 570 575

Pro Gly Lys Ala Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys Ala Gly  
580 585 590

Ser Cys Asp Leu Pro Gln Thr His Asn Leu Arg Asn Lys Arg Ala Leu  
595 600 605

Thr Leu Leu Val Gln Met Arg Arg Leu Ser Pro Leu Ser Cys Leu Lys  
610 615 620

Asp Arg Lys Asp Phe Gly Phe Pro Gln Glu Lys Val Asp Ala Gln Gln  
625 630 635 640

Ile Lys Ala Gln Ala Ile Pro Val Leu Ser Glu Leu Thr Gln Gln  
645 650 655

Ile Leu Asn Ile Phe Thr Ser Lys Asp Ser Ser Ala Ala Trp Asn Ala  
660 665 670

Thr Leu Leu Asp Ser Phe Cys Asn Asp Leu His Gln Gln Leu Asn Asp  
675 680 685

Leu Gln Gly Cys Leu Met Gln Gln Val Gly Val Gln Glu Phe Pro Leu  
690 695 700

Thr Gln Glu Asp Ala Leu Leu Ala Val Arg Lys Tyr Phe His Arg Ile  
705 710 715 720

Thr Val Tyr Leu Arg Glu Lys His Ser Pro Cys Ala Trp Glu Val  
725 730 735

Val Arg Ala Glu Val Trp Arg Ala Leu Ser Ser Ser Ala Asn Val Leu  
740 745 750

Gly Arg Leu Arg Glu Glu Lys  
755

<210> SEQ\_ID NO 9  
<211> LENGTH: 2251  
<212> TYPE: DNA  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Nucleic acid encoding fusion protein.

&lt;400&gt; SEQUENCE: 9

atgttacttgg	gactgaactg	tgtatcata	gtttttctct	taaaaagggtgt	ccagagtcatg	60
gtacacaactgc	agcagcctgg	ggctgagctg	gtgaagcctg	gggcctca	gt gaagatgtcc	120
tgcaaggctt	ctggctacac	atttaccagt	tacaatatgc	actgggttaaa	acagacacct	180
ggtcggggcc	ttggatggat	tggagctatt	tatcccggaa	atggtgatac	ttcctacaat	240
cagaagttca	aaggcaaggc	cacattgact	gcagacaat	cctccagcac	agcctacatg	300
cagctcagca	gcctgacatc	tgaggactct	gccccatatt	actgtgcaag	atcgacttac	360
tacggcggtg	actggtactt	caatgtctgg	ggcgccaggga	ccacggtcac	cgtctctgca	420

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gctagcacca	agggeccatc	ggttttcccc	ctggcgccct	gttccaggag	cacccctggg	480
ggcacacgcg	ccctgggctg	cctggtcaag	gactacttc	ccgaaccgg	gacggtgtcg	540
tggaaacttag	gcccctgac	cageggcgtg	cacacccccc	gggtgtct	acagtccca	600
ggactctact	ccctcagcag	cgtggtgacc	gtggccctcca	gcagcttggg	cacccagacc	660
tacacctgca	acgtgaatca	caagcccagc	aacaccaagg	tggacaaggag	agtttgtgag	720
aggccagcgc	agggagggag	ggtgtctgt	ggaagccagg	ctcageccctc	ctgcctggac	780
gcataccggc	tgtcagtc	cagccccagg	caccaaggca	ggcccccgtct	gactctcac	840
ccggaggcc	ctgcccggcc	cactcatgt	cagggagagg	gttttctggc	tttttccacc	900
aggctccggg	caggcacagg	ctggatgccc	ctaccccagg	cccttcacac	acaggggcag	960
gtgctgcgt	cagagctgcc	aagagccata	tccaggagga	ccctggccct	gaccgagctc	1020
aaaacccac	ttggtgacac	aactcacaca	tgcccacgg	gcccagagcc	caaatttgt	1080
gacacacctc	ccccgtgccc	aagggtgccc	gagccaaat	cttgtgacac	acccccccg	1140
tgcggccaa	gccccagagcc	caaattttgt	gacacacctc	ccccgtgccc	aagggtgccc	1200
tgatttcccg	gaccctgt	gtcacgtcg	tggtggtgga	cgtgagccac	gaagacccc	1260
aggtccagtt	caagtggta	gtggacggcg	tggaggtgca	taatgccaag	acaaagctgc	1320
gggaggagca	gtacaacacgc	acgttccgt	tggtcagcg	cctcaccgtc	ctgcaccagg	1380
actggctgaa	cggcaaggag	tacaagtgc	aggctctcaa	caaagccctc	ccagccccca	1440
tcgagaaacc	atctccaaag	ccaaaatgac	caagaacccag	gtcagcgt	cctgccttgg	1500
caaaggcttc	tacccagcg	acatgcccgt	ggagttggag	agcaatggc	agccggagaa	1560
caactacaac	accacgcctc	ccatgctgga	ctccgacggc	tccttttcc	tctacagcaa	1620
gctcaccgtg	gacaagagca	ggggcagca	ggggaaacatc	ttctcatgt	ccgtgatgca	1680
tgaggctctg	cacaaccact	acacgcgaa	gagcctctcc	ctgtctccgg	gtaaatctgg	1740
tgccgtgtgg	tcctgtgatc	tgcctcaaac	ccacagcgt	ggtagcagga	ggaccttgat	1800
gctcctggca	cagatgagga	gaatctctt	tttctctgc	ttgaaggaca	gacatgactt	1860
tgatattccc	caggaggagt	ttggcaacca	gttccaaagg	gtcggaaacca	tccctgtcct	1920
ccatgagatg	atccagcaga	tcttcaatct	cttcagcaca	aaggactcat	ctgctgctt	1980
ggatgagacc	ctcctagaca	aattctacac	tgaactctac	cagcagctga	atgacctgga	2040
agcctgtgt	atacaggggg	tgggggtgac	agagactccc	ctgatgaagg	aggactccat	2100
tctggctgt	aggaaatact	tccaaagaat	cactcttat	ctgaaaagaga	agaaaatacag	2160
cccttgc	tgggaggtt	tcagagcaga	aatcatgaga	tcttttctt	tgtcaacaaa	2220
cttgc	agaaagaa	agtttaagaa	gtaaggaat	a		2251

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&lt;210&gt; SEQ ID NO 10

&lt;211&gt; LENGTH: 750

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Fusion protein.

&lt;400&gt; SEQUENCE: 10

Met	Tyr	Leu	Gly	Leu	Asn	Cys	Val	Ile	Ile	Val	Phe	Leu	Leu	Lys	Gly
1															

5

10

15

Val	Gln	Ser	Gln	Val	Gln	Leu	Gln	Pro	Gly	Ala	Glu	Leu	Val	Lys
20														

25

30

Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe

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**67**

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**68**


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35	40	45
Thr Ser Tyr Asn Met His Trp Val Lys Gln Thr Pro Gly Arg Gly Leu		
50	55	60
Glu Trp Ile Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn		
65	70	75
Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser		
85	90	95
Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val		
100	105	110
Tyr Tyr Cys Ala Arg Ser Thr Tyr Gly Gly Asp Trp Tyr Phe Asn		
115	120	125
Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ala Ala Ser Thr Lys		
130	135	140
Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Gly		
145	150	155
160		
Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro		
165	170	175
Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr		
180	185	190
Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val		
195	200	205
Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Thr Cys Asn		
210	215	220
Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Gly Glu		
225	230	235
240		
Arg Pro Ala Gln Gly Gly Arg Val Ser Ala Gly Ser Gln Ala Gln Pro		
245	250	255
Ser Cys Leu Asp Ala Ser Arg Leu Cys Ser Pro Ser Pro Gly His Gln		
260	265	270
Gly Arg Pro Arg Leu Thr Pro His Pro Glu Ala Ser Ala Arg Pro Thr		
275	280	285
His Ala Gln Gly Glu Gly Leu Leu Ala Phe Ser Thr Arg Leu Arg Ala		
290	295	300
Gly Thr Gly Trp Met Pro Leu Pro Gln Ala Leu His Thr Gln Gly Gln		
305	310	315
320		
Val Leu Arg Ser Glu Leu Pro Arg Ala Ile Ser Arg Arg Thr Leu Pro		
325	330	335
Leu Thr Glu Leu Lys Thr Pro Leu Gly Asp Thr Thr His Thr Cys Pro		
340	345	350
Arg Cys Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg		
355	360	365
Cys Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys		
370	375	380
Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro		
385	390	395
400		
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser		
405	410	415
His Glu Asp Pro Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu		
420	425	430
Val His Asn Ala Lys Thr Lys Leu Arg Glu Glu Gln Tyr Asn Ser Thr		
435	440	445
Phe Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn		
450	455	460

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Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro  
465 470 475 480

Ile Glu Lys Thr Ile Ser Lys Ala Lys Met Thr Lys Asn Gln Val Ser  
485 490 495

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu  
500 505 510

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Asn Thr Thr Pro Pro  
515 520 525

Met Leu Asp Ser Asp Gly Ser Phe Leu Tyr Ser Lys Leu Thr Val  
530 535 540

Asp Lys Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met  
545 550 555 560

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser  
565 570 575

Pro Gly Lys Ser Gly Gly Gly Ser Cys Asp Leu Pro Gln Thr His  
580 585 590

Ser Leu Gly Ser Arg Arg Thr Leu Met Leu Leu Ala Gln Met Arg Arg  
595 600 605

Ile Ser Leu Phe Ser Cys Leu Lys Asp Arg His Asp Phe Gly Phe Pro  
610 615 620

Gln Glu Glu Phe Gly Asn Gln Phe Gln Lys Ala Glu Thr Ile Pro Val  
625 630 635 640

Leu His Glu Met Ile Gln Gln Ile Phe Asn Leu Phe Ser Thr Lys Asp  
645 650 655

Ser Ser Ala Ala Trp Asp Glu Thr Leu Leu Asp Lys Phe Tyr Thr Glu  
660 665 670

Leu Tyr Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Gly Val  
675 680 685

Gly Val Thr Glu Thr Pro Leu Met Lys Glu Asp Ser Ile Leu Ala Val  
690 695 700

Arg Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Lys Glu Lys Lys Tyr  
705 710 715 720

Ser Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe  
725 730 735

Ser Leu Ser Thr Asn Leu Gln Glu Ser Leu Arg Ser Lys Glu  
740 745 750

<210> SEQ\_ID NO 11  
<211> LENGTH: 2252  
<212> TYPE: DNA  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Nucleic acid encoding fusion protein.

&lt;400&gt; SEQUENCE: 11

atgttacttgg gactgaactg tgtaatcata gttttctct taaaagggtgt ccagagtcatg 60  
gtacaactgc agcagcctgg ggctgagctg gtgaagcctg gggcctcagt gaagatgtcc 120  
tgcaaggctt ctggctacac atttaccagt tacaatatgc actgggttaaa acagacacct 180  
ggtcggggcc tggaatggat tggagctatt tatcccgaa atggtgatac ttccctacaat 240  
cagaagttca aaggcaaggc cacattgact gcagacaaat cctccagcac agcctacatg 300  
cagctcagca gcctgacatc tgaggactct gcggtctatt actgtgcaag atcgacttac 360  
tacggcggtg actggtaactt caatgtctgg ggccgcaggaa ccacggtcac cgtctctgca 420

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gctagcacca	agggeccatc	ggttttcccc	ctggcgccct	gttccaggag	cacctctggg	480
ggcacagcg	ccctgggctg	cctggtaag	gactactcc	ccgaaccggt	gacggtgtcg	540
tggaaactcg	gcgcctgac	cageggcgtg	cacacccccc	cggtgtctt	acagtcccta	600
ggactctact	ccctcaggag	cgtggtgacc	gtggccctcc	gcagcttggg	cacccagacc	660
tacacctgca	acgtgaatca	caaggccccc	aacaccaagg	tggacaaggag	agttggtgag	720
aggccagcgc	agggaggggag	ggtgtctgt	ggaagccagg	ctcageccctc	ctgcctggac	780
gcataccggc	tgtcagtc	cagcccaagg	caccaaggca	ggcccccgtct	gactctcac	840
ccggaggcc	ctgcccggcc	cactcatgt	cagggagagg	gttttttcc	ttttttccacc	900
aggctccggg	caggcacagg	ctggatgccc	ctaccccaagg	cccttcacac	acaggggcag	960
gtgctgcgt	cagagctgcc	aagagccata	tccaggagga	ccctggccct	gaccgagctc	1020
aaaacccccac	ttggtgacac	aactcacaca	tgcccacgg	gcccaagagcc	caaatcttgt	1080
gacacacctc	ccccctgcccc	aagggtgccc	gagcccaaat	cttggacacac	accccccccg	1140
tgccccaaggt	gccccagagcc	caaatcttgt	gacacacctc	ccccctgcccc	aagggtgccc	1200
tgatttcccg	gaccctgtag	gtcacgtcg	tgggtggtag	cgtgagccac	gaagaccccg	1260
aggccatgtt	caagtggtag	gtggacggcg	tggaggtgca	taatgccaag	acaaagctgc	1320
gggaggagca	gtacaacacgc	acgttccgtg	tggtcagcg	cctcaccg	ctgcaccagg	1380
actggctgaa	cggcaaggag	tacaagtgc	aggtctccaa	caaagccctc	ccagccccca	1440
tccggaaaaac	catctccaaa	gccaaatga	ccaagaacca	ggtcagcc	acctgcctgg	1500
tcaaaggctt	ctacccca	gacatcgcc	tggagtgaa	gagcaatggg	cagccggaga	1560
acaactacaa	caccacgc	cccatgctgg	actccgacgg	ctcccttc	ctctacagca	1620
agtcacccgt	ggacaagagc	agggtggc	agggaaacat	cttctatgc	tccgtatgc	1680
atgaggctt	gcacaaccac	tacacgcaga	agagcctc	cctgtctcc	ggtaaatctg	1740
gtggcggtgg	atcctgtgat	ctgcctcaaa	cccacagcc	gggttagcagg	aggacattga	1800
tgcctctggc	acagatgagg	agaatctt	tttctctgt	tttgcaggac	agacatgact	1860
ttggatttcc	ccaggaggag	tttgcaacc	agttccaaa	ggctgaaacc	atcccgttcc	1920
tccatggat	gatccagcg	atcttc	tcttc	tctcagcac	aaaggactca	1980
gggatggac	cctccctagac	aaattctaca	ctgaactcta	ccagcagctg	aatgacccgtt	2040
aaggccgtgt	gatacagggg	gtgggggtga	cagagactcc	cctgatgaag	gaggactcca	2100
ttctgggtgt	gaggaaatac	ttccaaagaa	tcactctct	tctgaaagag	aagaaataca	2160
geccttgc	ctgggggtt	gtcagagcg	aatcatgag	atcttttct	ttgtcaacaa	2220
acttgc	aaga	aagttttaaga	agtaaggaat	ga		2252

&lt;210&gt; SEQ ID NO 12

&lt;211&gt; LENGTH: 757

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Fusion protein.

&lt;400&gt; SEQUENCE: 12

Met	Tyr	Leu	Gly	Leu	Asn	Cys	Val	Ile	Ile	Val	Phe	Leu	Leu	Lys	Gly
1				5				10				15			

Val	Gln	Ser	Gln	Val	Gln	Leu	Gln	Pro	Gly	Ala	Glu	Leu	Val	Lys
20				25								30		

Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe

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35	40	45
Thr Ser Tyr Asn Met His Trp Val Lys Gln Thr Pro Gly Arg Gly Leu		
50	55	60
Glu Trp Ile Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn		
65	70	75
80		
Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser		
85	90	95
Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val		
100	105	110
Tyr Tyr Cys Ala Arg Ser Thr Tyr Gly Gly Asp Trp Tyr Phe Asn		
115	120	125
Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ala Ala Ser Thr Lys		
130	135	140
Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Gly		
145	150	155
160		
Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro		
165	170	175
Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr		
180	185	190
Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val		
195	200	205
Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Thr Cys Asn		
210	215	220
Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Gly Glu		
225	230	235
240		
Arg Pro Ala Gln Gly Gly Arg Val Ser Ala Gly Ser Gln Ala Gln Pro		
245	250	255
Ser Cys Leu Asp Ala Ser Arg Leu Cys Ser Pro Ser Pro Gly His Gln		
260	265	270
Gly Arg Pro Arg Leu Thr Pro His Pro Glu Ala Ser Ala Arg Pro Thr		
275	280	285
His Ala Gln Gly Glu Gly Leu Leu Ala Phe Ser Thr Arg Leu Arg Ala		
290	295	300
Gly Thr Gly Trp Met Pro Leu Pro Gln Ala Leu His Thr Gln Gly Gln		
305	310	315
320		
Val Leu Arg Ser Glu Leu Pro Arg Ala Ile Ser Arg Arg Thr Leu Pro		
325	330	335
Leu Thr Glu Leu Lys Thr Pro Leu Gly Asp Thr Thr His Thr Cys Pro		
340	345	350
Arg Cys Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg		
355	360	365
Cys Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys		
370	375	380
Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro		
385	390	395
400		
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser		
405	410	415
His Glu Asp Pro Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu		
420	425	430
Val His Asn Ala Lys Thr Lys Leu Arg Glu Glu Gln Tyr Asn Ser Thr		
435	440	445
Phe Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn		
450	455	460

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Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro  
465 470 475 480

Ile Glu Lys Thr Ile Ser Lys Ala Lys Met Thr Lys Asn Gln Val Ser  
485 490 495

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu  
500 505 510

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Asn Thr Thr Pro Pro  
515 520 525

Met Leu Asp Ser Asp Gly Ser Phe Leu Tyr Ser Lys Leu Thr Val  
530 535 540

Asp Lys Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val Met  
545 550 555 560

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser  
565 570 575

Pro Gly Lys Ser Ala Glu Ala Ala Lys Glu Ala Ala Lys Ala  
580 585 590

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met  
595 600 605

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp  
610 615 620

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln  
625 630 635 640

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe  
645 650 655

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu  
660 665 670

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu  
675 680 685

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys  
690 695 700

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu  
705 710 715 720

Tyr Leu Lys Glu Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg  
725 730 735

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser  
740 745 750

Leu Arg Ser Lys Glu  
755

<210> SEQ ID NO 13  
<211> LENGTH: 1779  
<212> TYPE: DNA  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Nucleic acid encoding fusion protein.

&lt;400&gt; SEQUENCE: 13

atgtacttgg gactgaactg tgtaatcata gttttctct taaaagggtgt ccagagtca	60
gtacaactgc agcagcctgg ggctgagctg gtgaagcctg gggcctcagt gaagatgtcc	120
tgcaggcctt ctggctacac atttaccagt tacaatatgc actgggtaaa acagacacct	180
ggtcggggcc tggaaatggat tggagctatt tatcccgaaa atggtgatac ttccatcaat	240
cagaagttca aaggcaaggc cacattgact gcagacaaat cctccagcac agcctacatg	300
cagctcagca gcctgacatc tgaggactct gcgggtctatt actgtgcaag atcgacttac	360

tacggcggtg	actggtactt	caatgtctgg	ggcgcaggga	ccacggtcac	cgtctctgca	420
getagccaac	caagggccca	tcggcttccc	ccctggacc	ctcccttcaag	agcacctctg	480
ggggcacagc	ggccctgggc	tgccctggta	aggactactt	ccccgaaccg	ggagccaaa	540
tcttgtaca	aaactcacac	atgcccaccc	tgcccaatga	tctccggac	ccctgaggtc	600
acatgcgtgg	tggtggacgt	gagccacgaa	gaccctgagg	tcaagttcaa	ctggtacgtg	660
gacggcggtgg	aggtgcataa	tgccaagaca	aagccgcggg	aggagcagta	caacgcacg	720
taccgggtgg	tcagcgtctt	caccgtcctg	caccaggact	ggctgaatgg	caaggagtac	780
aagtgcagaag	tctccaacaa	agccctccca	gccccatcg	agaaaaccat	ctccaaagcc	840
aaagggtggga	cccggtgggt	gcgaggggca	catggacaga	ggccggctcg	gcccaccc	900
tgccctgaga	gtgaccgctg	taccaaccc	tgtcctacag	ggcagccccg	agaaccacag	960
gtgtacaccc	tgcccccate	ccgggatgag	ctgaccaaga	accaggtcag	cctgacctgc	1020
cgggtcaag	gttcttatcc	cagcgcacatc	gccgtggagt	gggagagcaa	tggcagccg	1080
gagaacaact	acaagaccac	gcctcccg	ctggactccg	acggctcctt	cttcctctac	1140
agcaagotca	ccgtggacaa	gagcaggtgg	cagcaggggg	acgtcttctc	atgctccgtg	1200
atgcatgagg	ctctgcacaa	ccactacacg	cagaagagcc	tctccctgtc	tccggtaaa	1260
tctgggtggcg	gtggatcctg	tgacetgcct	cagactcata	acctcaggaa	caagagagcc	1320
ttgacactcc	tggtacaaat	gaggagactc	tcccctctct	cctgcctgaa	ggacaggaag	1380
gactttggat	tcccgcagga	gaagggtggat	gcccagcaga	tcaagaaggc	tcaaggccatc	1440
cctgtctcta	gtgagctgac	ccagcagatc	ctgaacatct	tcacatcaa	ggactcatct	1500
gctgcttggaa	atgcaaccct	cctagactca	ttctgcaatg	acctccacca	gcagctcaat	1560
gacctgcaag	gttgtctgat	gcagcaggtg	gggggtgcagg	aatttccct	gaccaggaa	1620
gatgccctgc	tggctgtgag	gaaatacttc	cacaggatca	ctgtgtacct	gagagagaag	1680
aaacacagcc	cctgtgcctg	ggaggtggtc	agagcagaag	tctggagagc	cctgtctcc	1740
tctgccaatg	tgctggaaag	actgagagaa	gagaaatga			1779

&lt;210&gt; SEQ\_ID NO 14

&lt;211&gt; LENGTH: 592

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Fusion protein.

&lt;400&gt; SEQUENCE: 14

Met	Tyr	Leu	Gly	Leu	Asn	Cys	Val	Ile	Ile	Val	Phe	Leu	Leu	Lys	Gly
1							5		10				15		

Val	Gln	Ser	Gln	Val	Gln	Leu	Gln	Gln	Pro	Gly	Ala	Glu	Leu	Val	Lys
							20		25			30			

Pro	Gly	Ala	Ser	Val	Lys	Met	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe
						35		40			45				

Thr	Ser	Tyr	Asn	Met	His	Trp	Val	Lys	Gln	Thr	Pro	Gly	Arg	Gly	Leu
						50		55			60				

Glu	Trp	Ile	Gly	Ala	Ile	Tyr	Pro	Gly	Asn	Gly	Asp	Thr	Ser	Tyr	Asn
						65		70			75		80		

Gln	Lys	Phe	Lys	Gly	Lys	Ala	Thr	Leu	Thr	Ala	Asp	Lys	Ser	Ser	Ser
							85		90		95				

Thr	Ala	Tyr	Met	Gln	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val
							100		105		110				

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Tyr Tyr Cys Ala Arg Ser Thr Tyr Tyr Gly Gly Asp Trp Tyr Phe Asn  
115 120 125

Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ala Ala Ser Gln Pro  
130 135 140

Arg Ala His Arg Ser Ser Pro Trp His Pro Pro Pro Arg Ala Pro Leu  
145 150 155 160

Gly Ala Gln Arg Pro Trp Ala Ala Trp Ser Arg Thr Thr Ser Pro Asn  
165 170 175

Arg Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro  
180 185 190

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser  
195 200 205

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu  
210 215 220

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr  
225 230 235 240

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn  
245 250 255

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro  
260 265 270

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gly Thr Arg Gly Val Arg  
275 280 285

Gly Pro His Gly Gln Arg Pro Ala Arg Pro Thr Leu Cys Pro Glu Ser  
290 295 300

Asp Arg Cys Thr Asn Leu Cys Pro Thr Gly Gln Pro Arg Glu Pro Gln  
305 310 315 320

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val  
325 330 335

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val  
340 345 350

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro  
355 360 365

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr  
370 375 380

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val  
385 390 395 400

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu  
405 410 415

Ser Pro Gly Lys Ser Gly Gly Ser Cys Asp Leu Pro Gln Thr  
420 425 430

His Asn Leu Arg Asn Lys Arg Ala Leu Thr Leu Leu Val Gln Met Arg  
435 440 445

Arg Leu Ser Pro Leu Ser Cys Leu Lys Asp Arg Lys Asp Phe Gly Phe  
450 455 460

Pro Gln Glu Lys Val Asp Ala Gln Gln Ile Lys Lys Ala Gln Ala Ile  
465 470 475 480

Pro Val Leu Ser Glu Leu Thr Gln Gln Ile Leu Asn Ile Phe Thr Ser  
485 490 495

Lys Asp Ser Ser Ala Ala Trp Asn Ala Thr Leu Leu Asp Ser Phe Cys  
500 505 510

Asn Asp Leu His Gln Gln Leu Asn Asp Leu Gln Gly Cys Leu Met Gln  
515 520 525

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Gln Val Gly Val Gln Glu Phe Pro Leu Thr Gln Glu Asp Ala Leu Leu  
530 535 540

Ala Val Arg Lys Tyr Phe His Arg Ile Thr Val Tyr Leu Arg Glu Lys  
545 550 555 560

Lys His Ser Pro Cys Ala Trp Glu Val Val Arg Ala Glu Val Trp Arg  
565 570 575

Ala Leu Ser Ser Ser Ala Asn Val Leu Gly Arg Leu Arg Glu Glu Lys  
580 585 590

<210> SEQ ID NO 15  
<211> LENGTH: 1779  
<212> TYPE: DNA  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Nucleic acid encoding fusion protein.

<400> SEQUENCE: 15

atgtacttgg gactgaactg tgtaatcata gttttctct taaaaggtgt ccagagtcag	60
gtacaactgc agcagccctgg ggctgagctg gtgaagcctg gggcctcagt gaagatgtcc	120
tgcaaggcct ctggctacac atttaccagt tacaatatgc actgggtaaa acagacacct	180
ggtcggggcc tggaaatggat tggagctatt tatcccggaa atggtgatac ttccctacaat	240
cagaagttca aaggcaaggc cacattgact gcagacaaat cctccagcac agcctacatg	300
cagctcagca gcctgacatc tgaggactct gcggcttatt actgtgcaag atcgacttac	360
tacggcggtg actggtaactt caatgtctgg ggccgcaggaa ccacggtcac cgtctctgca	420
gctagccaac caagggccca tcggcttcc ccctggcacc ctccctcaag agcacctctg	480
ggggcacagc ggccctgggc tgcctggta aggactactt ccccgaaccg ggagccaaa	540
tcttgtaca aaactcacac atgcccaccc tgcccaatga tctccggac ccctgaggtc	600
acatgcgtgg tggtgacgt gagccacgaa gaccctgagg tcaagttcaa ctggtacgt	660
gacggcgtgg aggtgcataa tgccaagaca aagccgcggg aggagcagta caacagcacg	720
tacccgggtgg tcagcgtcct caccgtcctg caccaggact ggctgaatgg caaggagtag	780
aagtgcagg tctccaacaa agccccccca gccccatcg agaaaaccat ctccaaagcc	840
aaaggtggga cccgtggggt gcgaggggcca catggacaga ggccggctcg gcccaccc	900
tgcctgaga gtgaccgctg taccaaccc tgcctacag ggcagcccc agaaccacag	960
gtgtacaccc tgccccatc ccggatgag ctgaccaaga accaggtcag cctgaccc	1020
ctggcgtcaag gcttctatcc cagcgacatc gccgtggagt gggagagcaa tgggcagcc	1080
gagaacaact acaagaccac gcctccctg ctggactccg acggctccctt cttccctac	1140
agcaagctca ccgtggacaa gagcagggtgg cagcaggggg acgtttctc atgctccgt	1200
atgcacatgagg ctctgcacaa ccactacacg cagaagagcc tctccctgtc tccggtaaa	1260
tctgggtggcg gtggatcctg tgacccctgcct cagactcata acctcaggaa caagagagcc	1320
ttgacactcc tggtaaaaaat gaggagactc tcccctctc cctgcctgaa ggacagggaa	1380
gactttggat tcccgccagga gaagggtggat gcccagcaga tcaagaaggc tcaagccatc	1440
cctgtccctga gtgagctgac ccagcagatc ctgaacatct tcacatcaaa ggactcatct	1500
gctgcttggaa atgcaaccct cctagactca ttctgcaatg acctccacca gcagctcaat	1560
gacccctgcaag gttgtctgat gcagcagggtgg ggggtgcagg aatttccctt gacccaggaa	1620
gatgccctgc tggctgtgag gaaatacttc cacaggatca ctgtgtaccc gagagagaag	1680
aaacacagcc cctgtgcctg ggagggtggc agagcagaag tctggagagc cctgtctcc	1740

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tctgccaatg tgctggaaag actgagagaa gagaaatga 1779

<210> SEQ ID NO 16

<211> LENGTH: 599

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Fusion protein.

<400> SEQUENCE: 16

Met Tyr Leu Gly Leu Asn Cys Val Ile Ile Val Phe Leu Leu Lys Gly  
1 5 10 15

Val Gln Ser Gln Val Gln Leu Gln Pro Gly Ala Glu Leu Val Lys  
20 25 30

Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe  
35 40 45

Thr Ser Tyr Asn Met His Trp Val Lys Gln Thr Pro Gly Arg Gly Leu  
50 55 60

Glu Trp Ile Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn  
65 70 75 80

Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser  
85 90 95

Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val  
100 105 110

Tyr Tyr Cys Ala Arg Ser Thr Tyr Gly Gly Asp Trp Tyr Phe Asn  
115 120 125

Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ala Ala Ser Gln Pro  
130 135 140

Arg Ala His Arg Ser Ser Pro Trp His Pro Pro Pro Arg Ala Pro Leu  
145 150 155 160

Gly Ala Gln Arg Pro Trp Ala Ala Trp Ser Arg Thr Thr Ser Pro Asn  
165 170 175

Arg Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro  
180 185 190

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser  
195 200 205

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu  
210 215 220

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr  
225 230 235 240

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn  
245 250 255

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro  
260 265 270

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gly Thr Arg Gly Val Arg  
275 280 285

Gly Pro His Gly Gln Arg Pro Ala Arg Pro Thr Leu Cys Pro Glu Ser  
290 295 300

Asp Arg Cys Thr Asn Leu Cys Pro Thr Gly Gln Pro Arg Glu Pro Gln  
305 310 315 320

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val  
325 330 335

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val  
340 345 350

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Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro  
 355 360 365  
 Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr  
 370 375 380  
 Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val  
 385 390 395 400  
 Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu  
 405 410 415  
 Ser Pro Gly Lys Ser Ala Glu Ala Ala Lys Glu Ala Ala Ala Lys  
 420 425 430  
 Ala Cys Asp Leu Pro Gln Thr His Asn Leu Arg Asn Lys Arg Ala Leu  
 435 440 445  
 Thr Leu Leu Val Gln Met Arg Arg Leu Ser Pro Leu Ser Cys Leu Lys  
 450 455 460  
 Asp Arg Lys Asp Phe Gly Phe Pro Gln Glu Lys Val Asp Ala Gln Gln  
 465 470 475 480  
 Ile Lys Lys Ala Gln Ala Ile Pro Val Leu Ser Glu Leu Thr Gln Gln  
 485 490 495  
 Ile Leu Asn Ile Phe Thr Ser Lys Asp Ser Ser Ala Ala Trp Asn Ala  
 500 505 510  
 Thr Leu Leu Asp Ser Phe Cys Asn Asp Leu His Gln Gln Leu Asn Asp  
 515 520 525  
 Leu Gln Gly Cys Leu Met Gln Gln Val Gly Val Gln Glu Phe Pro Leu  
 530 535 540  
 Thr Gln Glu Asp Ala Leu Leu Ala Val Arg Lys Tyr Phe His Arg Ile  
 545 550 555 560  
 Thr Val Tyr Leu Arg Glu Lys Lys His Ser Pro Cys Ala Trp Glu Val  
 565 570 575  
 Val Arg Ala Glu Val Trp Arg Ala Leu Ser Ser Ser Ala Asn Val Leu  
 580 585 590  
 Gly Arg Leu Arg Glu Glu Lys  
 595

<210> SEQ ID NO 17  
 <211> LENGTH: 1776  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Nucleic acid encoding fusion protein.

<400> SEQUENCE: 17

atgtacttgg	gactgaactg	tgtaatcata	gtttttctct	taaaaggtgt	ccagagtcag	60
gtacaactgc	agcagcctgg	ggctgagctg	gtgaaggctg	gggcctca	gt gaatgtcc	120
tgcaggc	tttgcacac	atttaccagt	tacaatatgc	actgggtaaa	acagacac	180
ggtcggggcc	tggatggat	tggagctatt	tatccggaa	atggtgatac	ttcctacaat	240
cagaagg	tca aaggcaaggc	cacattgact	gcagacaat	cctccagcac	agcctacatg	300
cagctcg	ca gctgacatc	tgaggactct	gcccgttatt	actgtgcaag	atcgacttac	360
tacggcggt	actggta	ttt caatgtctgg	ggcgcaggaa	ccacggtcac	cgtctctgca	420
gttagcca	ac aaggccccca	tccgttcc	ccctggcacc	ctccctcaag	agcacctctg	480
ggggcac	gc acgcgggc	ttccgttcc	aggactactt	ccccgaacccg	ggageccaaa	540
tcttg	tgaca aactcacac	atgcccacccg	tgcccaatga	tctccggac	ccctgagg	600
acatgcgtt	ggtggacgt	gagccacgaa	gaccctgagg	tcaagttcaa	ctggta	660

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gacggcgtgg	aggtgtcataa	tgccaagaca	aagccgcggg	aggagcagta	caacagcacg	720
taccgggtgg	tcagcgctt	caccgtcctg	caccaggact	ggctgaatgg	caaggagtag	780
aagtgcagg	tctccaacaa	agccctccca	gccccatcg	agaaaaccat	ctccaaagcc	840
aaaggtggga	cccggtgggt	gcgagggcca	catggacaga	ggccggctcg	gcccaccctc	900
tgccctgaga	gtgaccgctg	taccaacctc	tgtcctacag	ggcagccccg	agaaccacag	960
gtgtacaccc	tgccccatc	ccgggatgag	ctgaccaaga	accaggatcg	cctgacacctc	1020
ctggtaaaag	gcttctatcc	cagggacatc	gccgtggagt	gggagagcaa	tggcagccg	1080
gagaacaact	acaagaccac	gcctccctg	ctggactccg	acggctccct	cttcctctac	1140
agcaagctca	ccgtggacaa	gagcaggggt	cagcagggga	acgtcttctc	atgctccgtg	1200
atgcatgagg	ctctgcacaa	ccactacacg	cagaagagcc	tctccctgtc	tccgggtaaa	1260
tctgggtggcg	gtggatcctg	tgtatctgcct	caaaccacaa	gcctggtag	caggaggacc	1320
ttgatgtcc	tggcacagat	gaggagaatc	tctctttct	cctgcttga	ggacagacat	1380
gactttggat	ttccccagga	ggagtttggc	aaccagttcc	aaaaggctga	aaccatccct	1440
gtcctccatg	agatgtatca	gcagatcttc	aatcttca	gcacaaagga	ctcatctgct	1500
gtttggatg	agaccctct	agacaaattc	tacactgaac	tctaccagca	gctgaatgac	1560
ctggaaaggct	gtgtgataca	gggggtgggg	gtgacagaga	ctcccctgat	gaaggaggac	1620
tccattctgg	ctgtgaggaa	atacttccaa	agaatcactc	tctatctgaa	agagaagaaa	1680
tacageccct	gtgcctggga	ggtgtcaga	gcagaaatca	ttagatctt	ttctttgtca	1740
acaaaacttgc	aagaaagttt	aagaagtaag	gaatga			1776

&lt;210&gt; SEQ ID NO 18

&lt;211&gt; LENGTH: 591

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Fusion protein.

&lt;400&gt; SEQUENCE: 18

Met	Tyr	Leu	Gly	Leu	Asn	Cys	Val	Ile	Ile	Val	Phe	Leu	Leu	Lys	Gly
1				5				10				15			

Val	Gln	Ser	Gln	Val	Gln	Leu	Gln	Pro	Gly	Ala	Glu	Leu	Val	Lys	
				20			25				30				

Pro	Gly	Ala	Ser	Val	Lys	Met	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe
				35			40				45				

Thr	Ser	Tyr	Asn	Met	His	Trp	Val	Lys	Gln	Thr	Pro	Gly	Arg	Gly	Leu
				50			55			60					

Glu	Trp	Ile	Gly	Ala	Ile	Tyr	Pro	Gly	Asn	Gly	Asp	Thr	Ser	Tyr	Asn
				65			70			75			80		

Gln	Lys	Phe	Lys	Gly	Lys	Ala	Thr	Leu	Thr	Ala	Asp	Lys	Ser	Ser	Ser
				85			90			95					

Thr	Ala	Tyr	Met	Gln	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val
				100			105			110					

Tyr	Tyr	Cys	Ala	Arg	Ser	Thr	Tyr	Tyr	Gly	Gly	Asp	Trp	Tyr	Phe	Asn
				115			120			125					

Val	Trp	Gly	Ala	Gly	Thr	Thr	Val	Thr	Val	Ser	Ala	Ala	Ser	Gln	Pro
				130			135			140					

Arg	Ala	His	Arg	Ser	Ser	Pro	Trp	His	Pro	Pro	Pro	Arg	Ala	Pro	Leu
				145			150			155			160		

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Gly Ala Gln Arg Pro Trp Ala Ala Trp Ser Arg Thr Thr Ser Pro Asn  
165 170 175

Arg Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro  
180 185 190

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser  
195 200 205

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu  
210 215 220

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr  
225 230 235 240

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn  
245 250 255

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro  
260 265 270

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gly Thr Arg Gly Val Arg  
275 280 285

Gly Pro His Gly Gln Arg Pro Ala Arg Pro Thr Leu Cys Pro Glu Ser  
290 295 300

Asp Arg Cys Thr Asn Leu Cys Pro Thr Gly Gln Pro Arg Glu Pro Gln  
305 310 315 320

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val  
325 330 335

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val  
340 345 350

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro  
355 360 365

Pro Val Leu Asp Ser Asp Gly Ser Phe Leu Tyr Ser Lys Leu Thr  
370 375 380

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val  
385 390 395 400

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu  
405 410 415

Ser Pro Gly Lys Ser Gly Gly Ser Cys Asp Leu Pro Gln Thr  
420 425 430

His Ser Leu Gly Ser Arg Arg Thr Leu Met Leu Leu Ala Gln Met Arg  
435 440 445

Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp Arg His Asp Phe Gly Phe  
450 455 460

Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln Lys Ala Glu Thr Ile Pro  
465 470 475 480

Val Leu His Glu Met Ile Gln Gln Ile Phe Asn Leu Phe Ser Thr Lys  
485 490 495

Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu Leu Asp Lys Phe Tyr Thr  
500 505 510

Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Gly  
515 520 525

Val Gly Val Thr Glu Thr Pro Leu Met Lys Glu Asp Ser Ile Leu Ala  
530 535 540

Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Lys Glu Lys Lys  
545 550 555 560

Tyr Ser Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser  
565 570 575

Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser Leu Arg Ser Lys Glu

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580

585

590

<210> SEQ ID NO 19  
<211> LENGTH: 1800  
<212> TYPE: PRT  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Fusion protein

<400> SEQUENCE: 19

Ala Thr Gly Thr Ala Cys Thr Thr Gly Gly Gly Ala Cys Thr Gly Ala  
1               5               10               15

Ala Cys Thr Gly Thr Gly Thr Ala Ala Thr Cys Ala Thr Ala Gly Thr  
20              25              30

Thr Thr Thr Cys Thr Cys Thr Ala Ala Ala Ala Gly Gly Thr  
35              40              45

Gly Thr Cys Cys Ala Gly Ala Gly Thr Cys Ala Gly Gly Thr Ala Cys  
50              55              60

Ala Ala Cys Thr Gly Cys Ala Gly Cys Ala Gly Cys Cys Thr Gly Gly  
65              70              75              80

Gly Gly Cys Thr Gly Ala Gly Cys Thr Gly Gly Thr Gly Ala Ala Gly  
85              90              95

Cys Cys Thr Gly Gly Cys Cys Thr Cys Ala Gly Thr Gly Ala  
100             105             110

Ala Gly Ala Thr Gly Thr Cys Cys Thr Gly Cys Ala Ala Gly Gly Cys  
115             120             125

Thr Thr Cys Thr Gly Gly Cys Thr Ala Cys Ala Cys Ala Thr Thr Thr  
130             135             140

Ala Cys Cys Ala Gly Thr Thr Ala Cys Ala Ala Thr Ala Thr Gly Cys  
145             150             155             160

Ala Cys Thr Gly Gly Thr Ala Ala Ala Cys Ala Gly Ala Cys  
165             170             175

Ala Cys Cys Thr Gly Gly Thr Cys Gly Gly Gly Cys Cys Thr Gly  
180             185             190

Gly Ala Ala Thr Gly Gly Ala Thr Thr Gly Gly Ala Gly Cys Thr Ala  
195             200             205

Thr Thr Thr Ala Thr Cys Cys Cys Gly Gly Ala Ala Ala Thr Gly Gly  
210             215             220

Thr Gly Ala Thr Ala Cys Thr Thr Cys Cys Thr Ala Cys Ala Ala Thr  
225             230             235             240

Cys Ala Gly Ala Ala Gly Thr Thr Cys Ala Ala Ala Gly Gly Cys Ala  
245             250             255

Ala Gly Gly Cys Cys Ala Cys Ala Thr Thr Gly Ala Cys Thr Gly Cys  
260             265             270

Ala Gly Ala Cys Ala Ala Ala Thr Cys Cys Thr Cys Cys Ala Gly Cys  
275             280             285

Ala Cys Ala Gly Cys Cys Thr Ala Cys Ala Thr Gly Cys Ala Gly Cys  
290             295             300

Thr Cys Ala Gly Cys Ala Gly Cys Cys Thr Gly Ala Cys Ala Thr Cys  
305             310             315             320

Thr Gly Ala Gly Ala Cys Thr Cys Thr Gly Cys Gly Gly Thr Cys  
325             330             335

Thr Ala Thr Thr Ala Cys Thr Gly Cys Ala Ala Gly Ala Thr  
340             345             350

Cys Gly Ala Cys Thr Thr Ala Cys Thr Ala Cys Gly Gly Cys Gly Gly

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355	360	365
Thr Gly Ala Cys Thr Gly Gly		
370	375	380
Thr Ala Cys Thr Thr Cys Ala Ala Thr		
Gly Thr Cys Thr Gly Gly Gly		
385	390	395
Gly Cys Ala Gly Gly Gly Cys Ala Gly Gly		
400		
Cys Cys Ala Cys Gly Gly Thr Cys Ala Cys Cys Gly		
405	410	415
Thr Cys Thr Cys Ala Cys Ala Ala Cys Cys Ala		
420	425	430
Ala Gly Gly Cys Cys Cys Ala Thr Cys Gly Gly		
435	440	445
Thr Cys Cys Cys Cys Thr Gly Gly Cys Ala Cys Cys Cys		
450	455	460
Thr Cys Cys Ala Ala Gly Ala Cys Ala Cys Thr Cys Thr Gly		
465	470	475
480		
Gly Gly Gly Cys Ala Cys Ala Gly Cys Gly Gly Cys Cys Cys		
485	490	495
Gly Gly Gly Cys Thr Gly Cys Cys Thr Gly Gly Thr Cys Ala Ala Gly		
500	505	510
Gly Ala Cys Thr Ala Cys Thr Thr Cys Cys Cys Gly Ala Ala Cys		
515	520	525
Cys Gly Gly Ala Gly Cys Cys Cys Ala Ala Ala Thr Cys Thr Thr		
530	535	540
Gly Thr Gly Ala Cys Ala Ala Ala Cys Thr Cys Ala Cys Ala Cys		
545	550	555
560		
Ala Thr Gly Cys Cys Ala Cys Cys Gly Thr Gly Cys Cys Ala		
565	570	575
Ala Thr Gly Ala Thr Cys Thr Cys Cys Gly Ala Cys Cys Cys		
580	585	590
Cys Thr Gly Ala Gly Gly Thr Cys Ala Cys Ala Thr Gly Cys Gly		
595	600	605
Gly Gly Thr Gly Gly Thr Gly Ala Cys Gly Thr Gly Ala Gly Cys		
610	615	620
Cys Ala Cys Gly Ala Ala Gly Ala Cys Cys Cys Thr Gly Ala Gly		
625	630	635
Gly		
640		
Thr Cys Ala Ala Gly Thr Thr Cys Ala Ala Cys Thr Gly Gly Thr Ala		
645	650	655
Cys Gly Thr Gly Ala Cys Gly Gly Cys Gly Thr Gly Ala Gly		
660	665	670
Gly Thr Gly Cys Ala Thr Ala Ala Thr Gly Cys Cys Ala Ala Gly		
675	680	685
Cys Ala Ala Ala Gly Cys Cys Gly Cys Gly Gly Ala Gly Gly Ala		
690	695	700
Gly Cys Ala Gly Thr Ala Cys Ala Ala Cys Ala Gly Cys Ala Cys		
705	710	715
Gly		
720		
Thr Ala Cys Cys Gly Gly Thr Gly Gly Thr Cys Ala Gly Cys Gly		
725	730	735
Thr Cys Cys Thr Cys Ala Cys Cys Gly Thr Cys Cys Thr Gly Cys Ala		
740	745	750
Cys Cys Ala Gly Gly Ala Cys Thr Gly Gly Cys Thr Gly Ala Ala Thr		
755	760	765
Gly Gly Cys Ala Ala Gly Gly Ala Gly Thr Ala Cys Ala Ala Gly Thr		
770	775	780

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Gly Cys Ala Ala Gly Gly Thr Cys Thr Cys Cys Ala Ala Cys Ala Ala  
 785 790 795 800

Ala Gly Cys Cys Cys Thr Cys Cys Ala Gly Cys Cys Cys Cys Cys  
 805 810 815

Ala Thr Cys Gly Ala Gly Ala Ala Ala Cys Cys Ala Thr Cys Thr  
 820 825 830

Cys Cys Ala Ala Ala Gly Cys Cys Ala Ala Ala Gly Gly Thr Gly Gly  
 835 840 845

Gly Ala Cys Cys Cys Gly Thr Gly Gly Gly Thr Gly Cys Gly Ala  
 850 855 860

Gly Gly Cys Cys Ala Cys Ala Thr Gly Gly Ala Cys Ala Gly Ala  
 865 870 875 880

Gly Gly Cys Cys Gly Gly Cys Thr Cys Gly Gly Cys Cys Cys Ala Cys  
 885 890 895

Cys Cys Thr Cys Thr Gly Cys Cys Thr Gly Ala Gly Ala Gly Thr  
 900 905 910

Gly Ala Cys Cys Gly Cys Thr Gly Thr Ala Cys Cys Ala Ala Cys Cys  
 915 920 925

Thr Cys Thr Gly Thr Cys Cys Thr Ala Cys Ala Gly Gly Cys Ala  
 930 935 940

Gly Cys Cys Cys Cys Gly Ala Gly Ala Ala Cys Cys Ala Cys Ala Gly  
 945 950 955 960

Gly Thr Gly Thr Ala Cys Ala Cys Cys Thr Gly Cys Cys Cys Cys  
 965 970 975

Cys Ala Thr Cys Cys Cys Gly Gly Ala Thr Gly Ala Gly Cys Thr  
 980 985 990

Gly Ala Cys Cys Ala Ala Gly Ala Ala Cys Cys Ala Gly Gly Thr Cys  
 995 1000 1005

Ala Gly Cys Cys Thr Gly Ala Cys Cys Thr Gly Cys Cys Thr Gly  
 1010 1015 1020

Gly Thr Cys Ala Ala Ala Gly Gly Cys Thr Thr Cys Thr Ala Thr  
 1025 1030 1035

Cys Cys Cys Ala Gly Cys Gly Ala Cys Ala Thr Cys Gly Cys Cys  
 1040 1045 1050

Gly Thr Gly Gly Ala Gly Thr Gly Gly Gly Ala Gly Ala Gly Cys  
 1055 1060 1065

Ala Ala Thr Gly Gly Cys Ala Gly Cys Cys Gly Gly Ala Gly  
 1070 1075 1080

Ala Ala Cys Ala Ala Cys Thr Ala Cys Ala Ala Gly Ala Cys Cys  
 1085 1090 1095

Ala Cys Gly Cys Cys Thr Cys Cys Cys Gly Thr Gly Cys Thr Gly  
 1100 1105 1110

Gly Ala Cys Thr Cys Cys Gly Ala Cys Gly Gly Cys Thr Cys Cys  
 1115 1120 1125

Thr Thr Cys Thr Thr Cys Cys Thr Cys Thr Ala Cys Ala Gly Cys  
 1130 1135 1140

Ala Ala Gly Cys Thr Cys Ala Cys Cys Gly Thr Gly Gly Ala Cys  
 1145 1150 1155

Ala Ala Gly Ala Gly Cys Ala Gly Gly Thr Gly Gly Cys Ala Gly  
 1160 1165 1170

Cys Ala Gly Gly Gly Ala Ala Cys Gly Thr Cys Thr Thr Cys  
 1175 1180 1185

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Thr Cys Ala Thr Gly Cys Thr Cys Cys Gly Thr Gly Ala Thr Gly  
 1190 1195 1200  
  
 Cys Ala Thr Gly Ala Gly Gly Cys Thr Cys Thr Gly Cys Ala Cys  
 1205 1210 1215  
  
 Ala Ala Cys Cys Ala Cys Thr Ala Cys Ala Cys Gly Cys Ala Gly  
 1220 1225 1230  
  
 Ala Ala Gly Ala Gly Cys Cys Thr Cys Thr Cys Cys Thr Gly  
 1235 1240 1245  
  
 Thr Cys Thr Cys Cys Gly Gly Thr Ala Ala Ala Gly Cys Ala  
 1250 1255 1260  
  
 Gly Ala Gly Gly Cys Cys Gly Cys Ala Gly Cys Thr Ala Ala Ala  
 1265 1270 1275  
  
 Gly Ala Gly Gly Cys Cys Gly Cys Ala Gly Cys Cys Ala Ala Ala  
 1280 1285 1290  
  
 Gly Cys Gly Gly Gly Ala Thr Cys Cys Thr Gly Thr Gly Ala Thr  
 1295 1300 1305  
  
 Cys Thr Gly Cys Cys Thr Cys Ala Ala Ala Cys Cys Cys Ala Cys  
 1310 1315 1320  
  
 Ala Gly Cys Cys Thr Gly Gly Thr Ala Gly Cys Ala Gly Gly  
 1325 1330 1335  
  
 Ala Gly Gly Ala Cys Cys Thr Thr Gly Ala Thr Gly Cys Thr Cys  
 1340 1345 1350  
  
 Cys Thr Gly Gly Cys Ala Cys Ala Gly Ala Thr Gly Ala Gly Gly  
 1355 1360 1365  
  
 Ala Gly Ala Ala Thr Cys Thr Cys Thr Cys Thr Thr Cys  
 1370 1375 1380  
  
 Thr Cys Cys Thr Gly Cys Thr Thr Gly Ala Ala Gly Gly Ala Cys  
 1385 1390 1395  
  
 Ala Gly Ala Cys Ala Thr Gly Ala Cys Thr Thr Thr Gly Gly Ala  
 1400 1405 1410  
  
 Thr Thr Thr Cys Cys Cys Ala Gly Gly Ala Gly Gly Ala Gly  
 1415 1420 1425  
  
 Thr Thr Thr Gly Gly Cys Ala Ala Cys Cys Ala Gly Thr Thr Cys  
 1430 1435 1440  
  
 Cys Ala Ala Ala Ala Gly Gly Cys Thr Gly Ala Ala Ala Cys Cys  
 1445 1450 1455  
  
 Ala Thr Cys Cys Cys Thr Gly Thr Cys Cys Thr Cys Cys Ala Thr  
 1460 1465 1470  
  
 Gly Ala Gly Ala Thr Gly Ala Thr Cys Cys Ala Gly Cys Ala Gly  
 1475 1480 1485  
  
 Ala Thr Cys Thr Thr Cys Ala Ala Thr Cys Thr Cys Thr Thr Cys  
 1490 1495 1500  
  
 Ala Gly Cys Ala Cys Ala Ala Ala Gly Gly Ala Cys Thr Cys Ala  
 1505 1510 1515  
  
 Thr Cys Thr Gly Cys Thr Gly Cys Thr Thr Gly Gly Gly Ala Thr  
 1520 1525 1530  
  
 Gly Ala Gly Ala Cys Cys Cys Thr Cys Cys Thr Ala Gly Ala Cys  
 1535 1540 1545  
  
 Ala Ala Ala Thr Thr Cys Thr Ala Cys Ala Cys Thr Gly Ala Ala  
 1550 1555 1560  
  
 Cys Thr Cys Thr Ala Cys Cys Ala Gly Cys Ala Gly Cys Thr Gly  
 1565 1570 1575  
  
 Ala Ala Thr Gly Ala Cys Cys Thr Gly Gly Ala Ala Gly Cys Cys

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**99****100**

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1580	1585	1590
Thr Gly	Thr Gly Thr Gly Ala	Thr Ala Cys Ala Gly
1595	1600	1605 Gly Gly Gly
Gly	Gly Gly Gly Gly	Gly Ala Cys Ala Gly Ala Gly
1610	1615	1620
Ala Cys	Thr Cys Cys Cys Cys	Thr Gly Ala Thr Gly Ala Ala Gly
1625	1630	1635
Gly Ala	Gly Ala Cys Thr Cys Cys Ala Thr Thr	Cys Thr Gly
1640	1645	1650
Gly Cys	Thr Gly Thr Gly Ala Gly	Gly Ala Ala Ala Thr Ala Cys
1655	1660	1665
Thr Thr	Cys Cys Ala Ala Ala	Gly Ala Ala Thr Cys Ala Cys Thr
1670	1675	1680
Cys Thr	Cys Thr Ala Thr Cys	Thr Gly Ala Ala Ala Gly Ala Gly
1685	1690	1695
Ala Ala	Gly Ala Ala Ala Thr Ala Cys Ala Gly Cys	Cys Cys Thr
1700	1705	1710
Thr Gly	Thr Gly Cys Cys Thr Gly	Gly Ala Gly Gly Thr Thr
1715	1720	1725
Gly Thr	Cys Ala Gly Ala Gly	Cys Ala Gly Ala Ala Ala Thr Cys
1730	1735	1740
Ala Thr	Gly Ala Gly Ala Thr Cys	Thr Thr Thr Thr Cys Thr
1745	1750	1755
Thr Thr	Gly Thr Cys Ala Ala Cys	Ala Ala Cys Thr Thr Gly
1760	1765	1770
Cys Ala	Ala Gly Ala Ala Ala Gly	Thr Thr Ala Ala Gly Ala
1775	1780	1785
Ala Gly	Thr Ala Ala Gly Gly	Ala Ala Thr Gly Ala
1790	1795	1800

<210> SEQ ID NO 20  
<211> LENGTH: 599  
<212> TYPE: PRT  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Fusion protein.

<400> SEQUENCE: 20

Met Tyr Leu Gly Leu Asn Cys Val Ile Ile Val Phe Leu Leu Lys Gly			
1	5	10	15
Val Gln Ser Gln Val Gln Leu Gln Pro Gly Ala Glu Leu Val Lys			
20	25	30	
Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe			
35	40	45	
Thr Ser Tyr Asn Met His Trp Val Lys Gln Thr Pro Gly Arg Gly Leu			
50	55	60	
Glu Trp Ile Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn			
65	70	75	80
Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser			
85	90	95	
Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val			
100	105	110	
Tyr Tyr Cys Ala Arg Ser Thr Tyr Tyr Gly Gly Asp Trp Tyr Phe Asn			
115	120	125	
Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ala Ala Ser Gln Pro			

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130	135	140
Arg Ala His Arg Ser Ser Pro Trp His Pro Pro Pro Arg Ala Pro Leu		
145	150	155
Gly Ala Gln Arg Pro Trp Ala Ala Trp Ser Arg Thr Thr Ser Pro Asn		
165	170	175
Arg Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro		
180	185	190
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser		
195	200	205
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu		
210	215	220
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr		
225	230	235
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn		
245	250	255
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro		
260	265	270
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gly Thr Arg Gly Val Arg		
275	280	285
Gly Pro His Gly Gln Arg Pro Ala Arg Pro Thr Leu Cys Pro Glu Ser		
290	295	300
Asp Arg Cys Thr Asn Leu Cys Pro Thr Gly Gln Pro Arg Glu Pro Gln		
305	310	315
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val		
325	330	335
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val		
340	345	350
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro		
355	360	365
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr		
370	375	380
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val		
385	390	395
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu		
405	410	415
Ser Pro Gly Lys Ala Glu Ala Ala Lys Glu Ala Ala Lys Ala		
420	425	430
Gly Ser Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr		
435	440	445
Leu Met Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu		
450	455	460
Lys Asp Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln		
465	470	475
Phe Gln Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln		
485	490	495
Ile Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu		
500	505	510
Thr Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp		
515	520	525
Leu Glu Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu		
530	535	540
Met Lys Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile		
545	550	555
		560

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Thr Leu Tyr Leu Lys Glu Lys Tyr Ser Pro Cys Ala Trp Glu Val  
565 570 575

Val Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln  
580 585 590

Glu Ser Leu Arg Ser Lys Glu  
595

<210> SEQ ID NO 21  
<211> LENGTH: 393  
<212> TYPE: DNA  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Nucleic acid encoding fusion protein.

<400> SEQUENCE: 21

atgggatgga	gctgggtaat	ccttttctc	ctgtcagtaa	ctgcagggtgt	ccactccag	60
tctgtgttga	cgcagccgccc	ctcagtgtct	gcccccccaag	gacagaagg	caccatctcc	120
tgcctctggaa	gcagtccaa	cattggaaat	aattatgttat	cctggtacca	gcagtccaa	180
ggaacagcccc	ccaaactcct	catctatgtat	cacaccaatc	ggcccgagg	ggtccctgac	240
cgattctctg	gctccaagtc	tggcacctca	gcctccctgg	ccatcagtgg	gttccgggtcc	300
gaggatgagg	ctgattatta	ctgtgcctcc	tgggactaca	ccctctcg	ctgggtgttc	360
ggaggaggga	ccaagggtcac	cgtccttaggt	gag			393

<210> SEQ ID NO 22

<211> LENGTH: 131  
<212> TYPE: PRT  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Fusion protein.

<400> SEQUENCE: 22

Met Gly Trp Ser Trp Val Ile Leu Phe Leu Leu Ser Val Thr Ala Gly  
1 5 10 15

Val His Ser Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Ala Ala  
20 25 30

Pro Gly Gln Lys Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile  
35 40 45

Gly Asn Asn Tyr Val Ser Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro  
50 55 60

Lys Leu Leu Ile Tyr Asp His Thr Asn Arg Pro Ala Gly Val Pro Asp  
65 70 75 80

Arg Phe Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser  
85 90 95

Gly Phe Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ser Trp Asp  
100 105 110

Tyr Thr Leu Ser Gly Trp Val Phe Gly Gly Thr Lys Val Thr Val  
115 120 125

Leu Gly Glu  
130

<210> SEQ ID NO 23  
<211> LENGTH: 1676  
<212> TYPE: DNA  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Nucleic acid encoding fusion protein.

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&lt;400&gt; SEQUENCE: 23

atgggatgga	gctgggtaat	gcacatttct	cctgtcagta	actgcagatg	cccgaaaag	60
gcctggagta	catggggctc	atctatcctg	gtgactctga	caccaaatac	agcccggtct	120
tccaaggcca	ggtaccatc	tcagtcgaca	agtccgtcag	cactgectac	ttgcaatgga	180
gcagtctgaa	gccctcggac	agcgcgtgt	atttttgtgc	gagacatgac	gtggatatt	240
gcaccgaccg	gacttgcgca	aagtggcctg	aatacttcca	gcattggggc	cagggcaccc	300
tggtcaccgt	ctcctcagct	agccaaccaa	gggcccacatcg	gtctcccccc	tggcacccctc	360
ctccaagagc	acactctgggg	gcacagcggc	cctgggtctg	ctggtcaagg	actacttccc	420
cgaaccggga	gccccaaatct	tgtgacaaaa	ctcacacatg	cccacccgtgc	ccaatgatct	480
cccgagcccc	tgaggtcaca	tgcggtgg	tggacgttag	ccacgaagac	cctgagggtca	540
agttcaactg	gtacgtggac	ggcgtggagg	tgcataatgc	caagacaaag	ccgcgggagg	600
agcagtacaa	cagcacgtac	cgggtggtca	gcgtcctcac	cgtcctgcac	caggactggc	660
tgaatggcaa	ggagttacaag	tgcaaggct	ccaacaaagc	cctcccagcc	cccatcgaga	720
aaaccatctc	caaagccaaa	ggtgggaccc	gtggggtgcg	agggccacat	ggacagaggc	780
cggctcgcc	caccctctgc	cctgagatgt	accgctgtac	caacctctgt	cctacagggc	840
agccccgaga	accacaggtg	tacaccctgc	ccccatcccg	ggatgagctg	accaagaacc	900
aggtcagcct	gaccctgcctg	gtcaaaggct	tctatcccag	cgacatcgcc	gtggagtgg	960
agagcaatgg	gcagccggag	aacaactaca	agaccacgc	tcccggtctg	gactccgacg	1020
gctccttctt	cctctacagc	aagctcacgg	tggacaagag	caggtggcag	caggggaacg	1080
tcttctcatg	ctccgtgatg	catgaggctc	tgccacaacca	ctacacgcag	aagacccct	1140
cctctgtcc	ggtaaatct	ggtggcggtg	gatcctgtga	cctgcctcag	actcataacc	1200
tcaggaacaa	gagagccttg	acactcctgg	tacaaatgag	gagactctcc	cctctctcct	1260
gcctgaagga	caggaaggac	tttggattcc	cgcaggagaa	ggtggatgcc	cagcagatca	1320
agaaggctca	agccatccct	gtcctgagtg	agctgaccca	gcagatcctg	aacatcttca	1380
catcaaagga	ctcatctgct	gcttggaaatg	caaccctcct	agactcatcc	tgcaatgacc	1440
tccaccagca	gctcaatgac	ctgcaagggt	gtctgatgca	gcaggtgggg	gtcaggaaat	1500
tccaccagca	ccaggaagat	gccctgctgg	ctgtgaggaa	atacttccac	aggatcactg	1560
tgtacctgag	agagaagaaa	cacagccct	gtgcctggg	ggtggatcaga	gcagaagtct	1620
ggagagccct	gtctccctct	gccaatgtgc	tggaaagact	gagagaagag	aaatga	1676

&lt;210&gt; SEQ ID NO 24

&lt;211&gt; LENGTH: 557

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Fusion protein.

&lt;400&gt; SEQUENCE: 24

Met	Gly	Trp	Ser	Trp	Val	Met	His	Leu	Ser	Pro	Val	Ser	Asn	Cys	Met
1				5		10			15						

Pro	Gly	Lys	Gly	Leu	Glu	Tyr	Met	Gly	Leu	Ile	Tyr	Pro	Gly	Asp	Ser
				20			25			30					

Asp	Thr	Lys	Tyr	Ser	Pro	Ser	Phe	Gln	Gly	Gln	Val	Thr	Ile	Ser	Val
					35		40		45						

Asp	Lys	Ser	Val	Ser	Thr	Ala	Tyr	Leu	Gln	Trp	Ser	Ser	Leu	Lys	Pro
					50		55		60						

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Ser Asp Ser Ala Val Tyr Phe Cys Ala Arg His Asp Val Gly Tyr Cys  
 65 70 75 80  
 Thr Asp Arg Thr Cys Ala Lys Trp Pro Glu Tyr Phe Gln His Trp Gly  
 85 90 95  
 Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Gln Pro Arg Ala His  
 100 105 110  
 Arg Ser Ser Pro Trp His Pro Pro Pro Arg Ala Pro Leu Gly Ala Gln  
 115 120 125  
 Arg Pro Trp Ala Ala Trp Ser Arg Thr Thr Ser Pro Asn Arg Glu Pro  
 130 135 140  
 Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Met Ile Ser  
 145 150 155 160  
 Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp  
 165 170 175  
 Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn  
 180 185 190  
 Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val  
 195 200 205  
 Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu  
 210 215 220  
 Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys  
 225 230 235 240  
 Thr Ile Ser Lys Ala Lys Gly Gly Thr Arg Gly Val Arg Gly Pro His  
 245 250 255  
 Gly Gln Arg Pro Ala Arg Pro Thr Leu Cys Pro Glu Ser Asp Arg Cys  
 260 265 270  
 Thr Asn Leu Cys Pro Thr Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr  
 275 280 285  
 Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr  
 290 295 300  
 Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu  
 305 310 315 320  
 Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu  
 325 330 335  
 Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys  
 340 345 350  
 Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu  
 355 360 365  
 Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly  
 370 375 380  
 Lys Ser Gly Gly Gly Ser Cys Asp Leu Pro Gln Thr His Asn Leu  
 385 390 395 400  
 Arg Asn Lys Arg Ala Leu Thr Leu Leu Val Gln Met Arg Arg Leu Ser  
 405 410 415  
 Pro Leu Ser Cys Leu Lys Asp Arg Lys Asp Phe Gly Phe Pro Gln Glu  
 420 425 430  
 Lys Val Asp Ala Gln Gln Ile Lys Lys Ala Gln Ala Ile Pro Val Leu  
 435 440 445  
 Ser Glu Leu Thr Gln Gln Ile Leu Asn Ile Phe Thr Ser Lys Asp Ser  
 450 455 460  
 Ser Ala Ala Trp Asn Ala Thr Leu Leu Asp Ser Phe Cys Asn Asp Leu  
 465 470 475 480

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His	Gln	Gln	Leu	Asn	Asp	Leu	Gln	Gly	Cys	Leu	Met	Gln	Gln	Val	Gly
485							490					495			

Val	Gln	Glu	Phe	Pro	Leu	Thr	Gln	Glu	Asp	Ala	Leu	Leu	Ala	Val	Arg
500							505					510			

Lys	Tyr	Phe	His	Arg	Ile	Thr	Val	Tyr	Leu	Arg	Glu	Lys	Lys	His	Ser
515							520				525				

Pro	Cys	Ala	Trp	Glu	Val	Val	Arg	Ala	Glu	Val	Trp	Arg	Ala	Leu	Ser
530							535				540				

Ser	Ser	Ala	Asn	Val	Leu	Gly	Arg	Leu	Arg	Glu	Glu	Lys			
545						550				555					

&lt;210&gt; SEQ ID NO 25

&lt;211&gt; LENGTH: 1700

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Nucleic acid encoding fusion protein.

&lt;400&gt; SEQUENCE: 25

atgggatgga	gctggtaat	gcacatttct	cctgtcagta	actgcagatg	cccgaaaag	60
gcctggaga	catggggctc	atctatcctg	gtgactctga	caccaaatac	agcccggtcct	120
tccaaggcca	ggtcaccatc	tcagtcgaca	agtccgttag	cactgcctac	ttgcaatgg	180
gcagtctgaa	gccctcgac	agcgcgtgt	atttttgc	gagacatgac	gtggatatt	240
gcaccgaccg	gacttgcgca	aagtggcctg	aataacttcca	gcattggggc	caggcaccc	300
tggtcaccgt	ctcctcagct	agccaaccaa	gggcacatcg	gtcttcccc	ttggcaccctc	360
ctccaagagc	acctctgggg	gcacagcgcc	cctgggtcgc	ctggtaagg	actacttccc	420
cgaaccggga	gcccaaatac	tgtgacaaaa	ctcacacatg	cccacccgtgc	ccaatgatct	480
cccgaccccc	tgaggtcaca	tgcggtgg	tggacgttag	ccacgaagac	cctgaggta	540
agttcaactg	gtacgtggac	ggcggtggagg	tgcataatgc	caagacaaag	ccgcgggagg	600
agcagtagcaa	cagcacgtac	cgggtggta	gcgtccctcac	cgtccctgcac	caggactggc	660
tgaatggcaa	ggagtacaag	tgcaaggct	ccaaacaaagc	cctcccaagcc	cccatcgaga	720
aaaccatctc	caaagccaaa	ggtgggaccc	gtgggggtcg	agggccacat	ggacagaggc	780
cggtctggcc	caccctctgc	cctgagatgt	accgctgtac	caacctctgt	cctacaggcc	840
agccccgaga	accacaggtg	tacaccctgc	cccatcccg	ggatgagctg	accaagaacc	900
aggtcagcct	gacctgcctg	gtcaaaggct	tctatcccg	cgacatcgcc	gtggagtgg	960
agagcaatgg	gcagccggag	aacaactaca	agaccacgac	tccctgtctg	gactccgacg	1020
gctccttctt	cctctacagc	aagctcacgg	tggacaagag	caggtggcag	cagggaaacg	1080
tcttctctatg	ctccgtatg	catgaggctc	tgcacaca	ctacacgcag	aagagccct	1140
ccctgtctcc	gggttaagca	gaggccgcag	ctaaagaggc	cgcagccaaa	gcgggatcc	1200
gtgacctgcc	tcaagactcat	aacctcagga	acaagagac	cttgacactc	ctggtaaaa	1260
tgaggagact	ctcccccttc	tcctgcctga	aggacaggaa	ggactttgg	ttcccgccagg	1320
agaagggtgga	tgcccgacag	atcaagaagg	ctcaagccat	cctgtcttg	agttagctga	1380
cccaggcagat	cctgaacatc	ttcacatca	aggactcatc	tgctgttgg	aatgcaccc	1440
tcctagactc	attctgcaat	gacctccacc	agcagctcaa	tgacctgca	ggttgtctga	1500
tgcagcaggt	gggggtgcag	gaatttcccc	tgacccagga	agatgcctg	ctggctgtga	1560
ggaaataactt	ccacaggatc	actgtgtacc	tgagagagaa	gaaacacagc	ccctgtgcct	1620

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gggaggtggt cagagcagaa gctctggagag ccctgttttc ctctgccaat gtgctggaa 1680  
 gactgagaga agagaaatga 1700

<210> SEQ ID NO 26  
 <211> LENGTH: 564  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Fusion protein.

<400> SEQUENCE: 26

Met	Gly	Trp	Ser	Trp	Val	Met	His	Leu	Ser	Pro	Val	Ser	Asn	Cys	Met
1						5			10					15	

Pro Gly Lys Gly Leu Glu Tyr Met Gly Leu Ile Tyr Pro Gly Asp Ser  
 20 25 30

Asp Thr Lys Tyr Ser Pro Ser Phe Gln Gly Gln Val Thr Ile Ser Val  
 35 40 45

Asp Lys Ser Val Ser Thr Ala Tyr Leu Gln Trp Ser Ser Leu Lys Pro  
 50 55 60

Ser Asp Ser Ala Val Tyr Phe Cys Ala Arg His Asp Val Gly Tyr Cys  
 65 70 75 80

Thr Asp Arg Thr Cys Ala Lys Trp Pro Glu Tyr Phe Gln His Trp Gly  
 85 90 95

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Gln Pro Arg Ala His  
 100 105 110

Arg Ser Ser Pro Trp His Pro Pro Pro Arg Ala Pro Leu Gly Ala Gln  
 115 120 125

Arg Pro Trp Ala Ala Trp Ser Arg Thr Thr Ser Pro Asn Arg Glu Pro  
 130 135 140

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Met Ile Ser  
 145 150 155 160

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp  
 165 170 175

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn  
 180 185 190

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val  
 195 200 205

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu  
 210 215 220

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys  
 225 230 235 240

Thr Ile Ser Lys Ala Lys Gly Gly Thr Arg Gly Val Arg Gly Pro His  
 245 250 255

Gly Gln Arg Pro Ala Arg Pro Thr Leu Cys Pro Glu Ser Asp Arg Cys  
 260 265 270

Thr Asn Leu Cys Pro Thr Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr  
 275 280 285

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr  
 290 295 300

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu  
 305 310 315 320

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu  
 325 330 335

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys  
 340 345 350

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Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu  
355 360 365

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly  
370 375 380

Lys Ser Ala Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys Ala Cys Asp  
385 390 395 400

Leu Pro Gln Thr His Asn Leu Arg Asn Lys Arg Ala Leu Thr Leu Leu  
405 410 415

Val Gln Met Arg Arg Leu Ser Pro Leu Ser Cys Leu Lys Asp Arg Lys  
420 425 430

Asp Phe Gly Phe Pro Gln Glu Lys Val Asp Ala Gln Gln Ile Lys Lys  
435 440 445

Ala Gln Ala Ile Pro Val Leu Ser Glu Leu Thr Gln Gln Ile Leu Asn  
450 455 460

Ile Phe Thr Ser Lys Asp Ser Ser Ala Ala Trp Asn Ala Thr Leu Leu  
465 470 475 480

Asp Ser Phe Cys Asn Asp Leu His Gln Gln Leu Asn Asp Leu Gln Gly  
485 490 495

Cys Leu Met Gln Gln Val Gly Val Gln Glu Phe Pro Leu Thr Gln Glu  
500 505 510

Asp Ala Leu Leu Ala Val Arg Lys Tyr Phe His Arg Ile Thr Val Tyr  
515 520 525

Leu Arg Glu Lys Lys His Ser Pro Cys Ala Trp Glu Val Val Arg Ala  
530 535 540

Glu Val Trp Arg Ala Leu Ser Ser Ser Ala Asn Val Leu Gly Arg Leu  
545 550 555 560

Arg Glu Glu Lys

<210> SEQ\_ID NO 27  
<211> LENGTH: 1673  
<212> TYPE: DNA  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Nucleic acid encoding fusion protein.

<400> SEQUENCE: 27

atggggatgga	gctgggtaat	gcacatttct	cctgtcagta	actgcagatg	cccgaaaag	60
gcctggagta	catggggctc	atctatcctg	gtgactctga	caccaaatac	agcccgctct	120
tccaaggcca	ggtcaccatc	tcagtcgaca	agtccgtca	cactgcctac	ttgcaatgga	180
geagtctgaa	gccctcgac	agcgcgtgt	atttttgc	gagacatgac	gtggatatt	240
geaccgcacg	gacttgcgca	aagtggctg	aataacttcca	gcattgggc	cagggcaccc	300
tggtcaccgt	ctcctcagct	agccaaccaa	gggcccacatcg	gtttcccc	tggcacccctc	360
ctccaagagc	acctctgggg	gcacagcgcc	cctgggtctgc	ctggtaagg	actacttccc	420
cgaaccggga	gcccaaatct	tgtgacaaaa	ctcacacatg	cccacccgtgc	ccaatgatct	480
cccgggacccc	tgaggtcaca	tgctgtgtgg	tggacgttag	ccacgaagac	cctgagggtca	540
agttcaactg	gtacgtggac	ggcgtggagg	tgcataatgc	caagacaaag	ccgcgggagg	600
agcagtacaa	cagcacgtac	cgggtggta	gcgtcctcac	cgtcctgcac	caggactggc	660
tgaatggcaa	ggagtacaag	tgcaaggct	ccaaacaaagc	cctcccaagcc	cccatcgaga	720
aaaccatctc	caaagccaaa	ggtgggaccc	gtgggggtgcg	agggccacat	ggacagaggc	780
cggctcgccc	caccctctgc	cctgagagtg	accgctgtac	caacctctgt	cctacagggc	840

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agccccgaga accacaggtg tacaccctgc ccccatcccg ggatgagctg accaagaacc	900
aggtcagect gacactgcctg gtcaaaggct tctatcccag cgacatcgcc gtggagtggg	960
agagcaatgg gcagccggag aacaactaca agaccacgcc tcccggtctg gactccgacg	1020
gtcccttctt cctctacagc aagctcacccg tggacaagag caggtggcag caggggaacg	1080
tcttctcatg ctccgtgatg catgaggctc tgccacaacca ctacacgcag aagagctct	1140
cctctgtcc gggtaaatct ggtggcggtg gatcctgtga tctgcctcaa acccacagcc	1200
tggtagcag gaggacattg atgtccttg cacagatgag gagaatctct ttttctct	1260
gettgaagga cagacatgac tttggatttc cccaggagga gtttggcaac cagttccaaa	1320
aggctgaaac catccctgtc ctccatgaga tgatccagca gatcttcaat ctctttagca	1380
caaaggactc atctgctgct tgggtatgaga ccctcctaga caaattctac actgaactct	1440
accagcagct gaatgacactg gaagcctgtg tgatacaggg ggtgggggtg acagagactc	1500
cctgtatgaa ggaggactcc attctggctg tgaggaaata cttccaaaga atcactct	1560
atctgaaaga gaagaaatac agcccttgtg cctggaggt tgtcagagca gaaatcatga	1620
gatcttttc ttgtcaaca aacttgcaag aaagtttaag aagtaaggaa tga	1673

&lt;210&gt; SEQ ID NO 28

&lt;211&gt; LENGTH: 591

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Fusion protein.

&lt;400&gt; SEQUENCE: 28

Met Tyr Leu Gly Leu Asn Cys Val Ile Ile Val Phe Leu Leu Lys Gly			
1	5	10	15

Val Gln Ser Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys			
20	25	30	

Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe			
35	40	45	

Thr Ser Tyr Asn Met His Trp Val Lys Gln Thr Pro Gly Arg Gly Leu			
50	55	60	

Glu Trp Ile Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn			
65	70	75	80

Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser			
85	90	95	

Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val			
100	105	110	

Tyr Tyr Cys Ala Arg Ser Thr Tyr Tyr Gly Gly Asp Trp Tyr Phe Asn			
115	120	125	

Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ala Ala Ser Gln Pro			
130	135	140	

Arg Ala His Arg Ser Ser Pro Trp His Pro Pro Pro Arg Ala Pro Leu			
145	150	155	160

Gly Ala Gln Arg Pro Trp Ala Ala Trp Ser Arg Thr Thr Ser Pro Asn			
165	170	175	

Arg Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro			
180	185	190	

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser			
195	200	205	

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu

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210	215	220
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr		
225	230	235
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn		
245	250	255
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro		
260	265	270
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gly Thr Arg Gly Val Arg		
275	280	285
Gly Pro His Gly Gln Arg Pro Ala Arg Pro Thr Leu Cys Pro Glu Ser		
290	295	300
Asp Arg Cys Thr Asn Leu Cys Pro Thr Gly Gln Pro Arg Glu Pro Gln		
305	310	315
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val		
325	330	335
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val		
340	345	350
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro		
355	360	365
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr		
370	375	380
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val		
385	390	395
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu		
405	410	415
Ser Pro Gly Lys Ser Gly Gly Ser Cys Asp Leu Pro Gln Thr		
420	425	430
His Ser Leu Gly Ser Arg Arg Thr Leu Met Leu Leu Ala Gln Met Arg		
435	440	445
Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp Arg His Asp Phe Gly Phe		
450	455	460
Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln Lys Ala Glu Thr Ile Pro		
465	470	475
Val Leu His Glu Met Ile Gln Gln Ile Phe Asn Leu Phe Ser Thr Lys		
485	490	495
Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu Leu Asp Lys Phe Tyr Thr		
500	505	510
Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Gly		
515	520	525
Val Gly Val Thr Glu Thr Pro Leu Met Lys Glu Asp Ser Ile Leu Ala		
530	535	540
Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Lys Glu Lys Lys		
545	550	555
Tyr Ser Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser		
565	570	575
Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser Leu Arg Ser Lys Glu		
580	585	590

<210> SEQ ID NO 29  
<211> LENGTH: 1697  
<212> TYPE: DNA  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Nucleic acid encoding fusion protein.

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&lt;400&gt; SEQUENCE: 29

atggggatgga	gctgggtaat	gcacatttct	cctgtcagta	actgcagatg	cccgaaaag	60
gcctggagta	catggggctc	atctatcctg	gtgactctga	caccaaatac	agcccgctt	120
tccaaggcca	ggtcaccatc	tcagtcgaca	agtccgttag	cactgectac	ttgcaatgga	180
gcagtcgtaa	gccctcgac	agcgcgtgt	atttttgtgc	gagacatgac	gtggatatt	240
gcaccgaccg	gacttgcgca	aagtggctg	aatacttcca	gcattggggc	cagggcaccc	300
tggtcaccgt	ctcctcagct	agccaaccaa	gggcccacatcg	gtctcccc	tggcaccc	360
ctccaagagc	acctctgggg	gcacagcgcc	cctgggtgc	ctggtaaagg	actacttccc	420
cgaaccggga	gccccaaatct	tgtgacaaaa	ctcacacatg	cccacccgtgc	ccaaatgatct	480
cccgggacccc	tgaggtcaca	tgcggtgg	tggacgttag	ccacgaagac	cctgaggta	540
agttcaactg	gtacgtggac	ggcgtggagg	tgcataatgc	caagacaaag	ccgcgggagg	600
agcagtacaa	cagcacgtac	cgggtggta	gcgtcctcac	cgtcctgcac	caggactggc	660
tgaatggcaa	ggagttacaag	tgcaaggct	ccaacaaagc	cctcccaagcc	cccatcgaga	720
aaaccatctc	caaagccaaa	ggtgggaccc	gtgggggtgcg	agggccacat	ggacagaggc	780
cggctcgccc	caccctctgc	cctgagatgt	accgctgtac	caacctctgt	cctacaggc	840
agccccgaga	accacaggtg	tacaccctgc	ccccatcccg	ggatgagctg	accaagaacc	900
aggtcagcct	gaccctgcctg	gtcaaaggct	tctatccag	cgacatcgcc	gtggagtgg	960
agagcaatgg	gcagccggag	aacaactaca	agaccacgac	tcccggtctg	gactccgacg	1020
gctccttctt	cctctcagc	aagctcaccg	tggacaagag	caggtggcag	caggggaacg	1080
tcttctcatg	ctccgtatg	catgaggctc	tgcacaacca	ctacacgcag	aagacccct	1140
cctctgtccc	gggttaagca	gaggccgcag	ctaaagggc	cgcagccaa	gcgggatcc	1200
tgatctgcc	tcaaaccac	agcctggta	gcaggaggac	cttgatgctc	ctggcacaga	1260
tgaggagaat	ctctttttc	tcctgcttga	aggacagaca	tgactttgga	tttccccagg	1320
aggagtttg	caaccagttc	caaaaggctg	aaaccatccc	tgtctccat	gagatgatcc	1380
agcagatctt	caatctttc	agcacaaagg	actcatctgc	tgtttggat	gagaccctcc	1440
tagacaaatt	ctacactgaa	ctctaccaggc	agctgaatga	cctggaaagcc	tgtgtgatac	1500
aggggggtggg	ggtgacagag	actccctga	tgaaggagga	ctccattctg	gctgtgagga	1560
aatacttcca	aagaatcact	ctctatctga	aagagaagaa	atacagccct	tgtgcctgg	1620
aggttgcag	agcagaaaatc	atgagatctt	tttctttgtc	aacaaacttg	caagaaagtt	1680
taagaagtaa	ggaatga					1697

&lt;210&gt; SEQ ID NO 30

&lt;211&gt; LENGTH: 598

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Fusion protein.

&lt;400&gt; SEQUENCE: 30

Met	Tyr	Leu	Gly	Leu	Asn	Cys	Val	Ile	Ile	Val	Phe	Leu	Lys	Gly
1							5			10			15	

Val	Gln	Ser	Gln	Val	Gln	Leu	Gln	Gln	Pro	Gly	Ala	Glu	Leu	Val	Lys
							20			25			30		

Pro	Gly	Ala	Ser	Val	Lys	Met	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe
							35			40			45		

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Thr Ser Tyr Asn Met His Trp Val Lys Gln Thr Pro Gly Arg Gly Leu  
 50 55 60

Glu Trp Ile Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn  
 65 70 75 80

Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser  
 85 90 95

Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val  
 100 105 110

Tyr Tyr Cys Ala Arg Ser Thr Tyr Gly Gly Asp Trp Tyr Phe Asn  
 115 120 125

Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ala Ala Ser Gln Pro  
 130 135 140

Arg Ala His Arg Ser Ser Pro Trp His Pro Pro Pro Arg Ala Pro Leu  
 145 150 155 160

Gly Ala Gln Arg Pro Trp Ala Ala Trp Ser Arg Thr Thr Ser Pro Asn  
 165 170 175

Arg Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro  
 180 185 190

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser  
 195 200 205

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu  
 210 215 220

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr  
 225 230 235 240

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn  
 245 250 255

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro  
 260 265 270

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gly Thr Arg Gly Val Arg  
 275 280 285

Gly Pro His Gly Gln Arg Pro Ala Arg Pro Thr Leu Cys Pro Glu Ser  
 290 295 300

Asp Arg Cys Thr Asn Leu Cys Pro Thr Gly Gln Pro Arg Glu Pro Gln  
 305 310 315 320

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val  
 325 330 335

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val  
 340 345 350

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro  
 355 360 365

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr  
 370 375 380

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val  
 385 390 395 400

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu  
 405 410 415

Ser Pro Gly Lys Ser Ala Glu Ala Ala Lys Glu Ala Ala Ala Lys  
 420 425 430

Ala Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu  
 435 440 445

Met Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys  
 450 455 460

Asp Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe

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465	470	475	480
Gln Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile			
485	490	495	
Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr			
500	505	510	
Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu			
515	520	525	
Glu Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met			
530	535	540	
Lys Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr			
545	550	555	560
Leu Tyr Leu Lys Glu Lys Tyr Ser Pro Cys Ala Trp Glu Val Val			
565	570	575	
Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu			
580	585	590	
Ser Leu Arg Ser Lys Glu			
595			

<210> SEQ ID NO 31  
<211> LENGTH: 15  
<212> TYPE: PRT  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Peptide linker.

&lt;400&gt; SEQUENCE: 31

Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser
1			5		10		15					

<210> SEQ ID NO 32  
<211> LENGTH: 5  
<212> TYPE: PRT  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Peptide linker.

&lt;400&gt; SEQUENCE: 32

Gly	Gly	Gly	Gly	Ser
1			5	

<210> SEQ ID NO 33  
<211> LENGTH: 7  
<212> TYPE: PRT  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Peptide linker.

&lt;400&gt; SEQUENCE: 33

Ala	Glu	Ala	Ala	Ala	Lys	Ala
1				5		

<210> SEQ ID NO 34  
<211> LENGTH: 12  
<212> TYPE: PRT  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Peptide linker

&lt;400&gt; SEQUENCE: 34

Ala	Glu	Ala	Ala	Ala	Lys	Glu	Ala	Ala	Lys	Ala
1				5			10			

-continued

<210> SEQ ID NO 35  
<211> LENGTH: 17  
<212> TYPE: PRT  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Peptide linker

<400> SEQUENCE: 35

Ala Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys  
1               5               10               15

Ala

<210> SEQ ID NO 36  
<211> LENGTH: 22  
<212> TYPE: PRT  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Peptide linker

<400> SEQUENCE: 36

Ala Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys  
1               5               10               15

Glu Ala Ala Ala Lys Ala  
20

<210> SEQ ID NO 37  
<211> LENGTH: 27  
<212> TYPE: PRT  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Peptide linker

<400> SEQUENCE: 37

Ala Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys  
1               5               10               15

Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys Ala  
20               25

<210> SEQ ID NO 38  
<211> LENGTH: 7  
<212> TYPE: PRT  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Peptide linker.

<400> SEQUENCE: 38

Ala Glu Ala Ala Ala Lys Ala  
1               5

<210> SEQ ID NO 39  
<211> LENGTH: 5  
<212> TYPE: PRT  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Peptide linker.

<400> SEQUENCE: 39

Gly Gly Gly Gly Gly  
1               5

<210> SEQ ID NO 40  
<211> LENGTH: 8  
<212> TYPE: PRT  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Peptide linker.

-continued

&lt;400&gt; SEQUENCE: 40

Gly Gly Gly Gly Gly Gly Gly  
1               5

&lt;210&gt; SEQ ID NO 41

&lt;211&gt; LENGTH: 5

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Peptide linker.

&lt;400&gt; SEQUENCE: 41

Gly Gly Ala Gly Gly  
1               5

&lt;210&gt; SEQ ID NO 42

&lt;211&gt; LENGTH: 10

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Peptide linker.

&lt;400&gt; SEQUENCE: 42

Gly Ala Gly Ala Gly Ala Gly Ala Gly Ala  
1               5               10

&lt;210&gt; SEQ ID NO 43

&lt;211&gt; LENGTH: 18

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Peptide linker.

&lt;400&gt; SEQUENCE: 43

Arg Pro Leu Ser Tyr Arg Pro Pro Phe Pro Phe Gly Phe Pro Ser Val  
1               5               10               15

Arg Pro

&lt;210&gt; SEQ ID NO 44

&lt;211&gt; LENGTH: 18

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Peptide linker.

&lt;400&gt; SEQUENCE: 44

Tyr Pro Arg Ser Ile Tyr Ile Arg Arg Arg His Pro Ser Pro Ser Leu  
1               5               10               15

Thr Thr

&lt;210&gt; SEQ ID NO 45

&lt;211&gt; LENGTH: 18

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Peptide linker.

&lt;400&gt; SEQUENCE: 45

Thr Pro Ser His Leu Ser His Ile Leu Pro Ser Phe Gly Leu Pro Thr  
1               5               10               15

Phe Asn

&lt;210&gt; SEQ ID NO 46

-continued

<211> LENGTH: 18  
<212> TYPE: PRT  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Peptide linker.

&lt;400&gt; SEQUENCE: 46

Arg	Pro	Val	Ser	Pro	Phe	Thr	Phe	Pro	Arg	Leu	Ser	Asn	Ser	Trp	Leu
1				5				10						15	

Pro Ala

<210> SEQ ID NO 47  
<211> LENGTH: 18  
<212> TYPE: PRT  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Peptide linker.

&lt;400&gt; SEQUENCE: 47

Ser	Pro	Ala	Ala	His	Phe	Pro	Arg	Ser	Ile	Pro	Arg	Pro	Gly	Pro	Ile
1				5				10					15		

Arg Thr

<210> SEQ ID NO 48  
<211> LENGTH: 18  
<212> TYPE: PRT  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Peptide linker.

&lt;400&gt; SEQUENCE: 48

Ala	Pro	Gly	Pro	Ser	Ala	Pro	Ser	His	Arg	Ser	Leu	Pro	Ser	Arg	Ala
1				5				10					15		

Phe Gly

<210> SEQ ID NO 49  
<211> LENGTH: 18  
<212> TYPE: PRT  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Peptide linker.

&lt;400&gt; SEQUENCE: 49

Pro	Arg	Asn	Ser	Ile	His	Phe	Leu	His	Pro	Leu	Leu	Val	Ala	Pro	Leu
1				5				10					15		

Gly Ala

<210> SEQ ID NO 50  
<211> LENGTH: 18  
<212> TYPE: PRT  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Peptide linker.

&lt;400&gt; SEQUENCE: 50

Met	Pro	Ser	Leu	Ser	Gly	Val	Leu	Gln	Val	Arg	Tyr	Leu	Ser	Pro	Pro
1					5			10					15		

Asp Leu

<210> SEQ ID NO 51  
<211> LENGTH: 18  
<212> TYPE: PRT  
<213> ORGANISM: Artificial  
<220> FEATURE:

-continued

&lt;223&gt; OTHER INFORMATION: Peptide linker.

&lt;400&gt; SEQUENCE: 51

Ser	Pro	Gln	Tyr	Pro	Ser	Pro	Leu	Thr	Leu	Thr	Leu	Pro	Pro	His	Pro
1															
							5					10			15

Ser Leu

&lt;210&gt; SEQ ID NO 52

&lt;211&gt; LENGTH: 18

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Peptide linker.

&lt;400&gt; SEQUENCE: 52

Asn	Pro	Ser	Leu	Asn	Pro	Pro	Ser	Tyr	Leu	His	Arg	Ala	Pro	Ser	Arg
1															
								5		10			15		

Ile Ser

&lt;210&gt; SEQ ID NO 53

&lt;211&gt; LENGTH: 17

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Peptide linker.

&lt;400&gt; SEQUENCE: 53

Leu	Pro	Trp	Arg	Thr	Ser	Leu	Leu	Pro	Ser	Leu	Pro	Leu	Arg	Arg	Arg
1															
								5		10			15		

Pro

&lt;210&gt; SEQ ID NO 54

&lt;211&gt; LENGTH: 18

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Peptide linker.

&lt;400&gt; SEQUENCE: 54

Pro	Pro	Leu	Phe	Ala	Lys	Gly	Pro	Val	Gly	Leu	Leu	Ser	Arg	Ser	Phe
1															
								5		10			15		

Pro Pro

&lt;210&gt; SEQ ID NO 55

&lt;211&gt; LENGTH: 18

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Peptide linker.

&lt;400&gt; SEQUENCE: 55

Val	Pro	Pro	Ala	Pro	Val	Val	Ser	Leu	Arg	Ser	Ala	His	Ala	Arg	Pro
1															
								5		10			15		

Pro Tyr

&lt;210&gt; SEQ ID NO 56

&lt;211&gt; LENGTH: 17

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Peptide linker.

&lt;400&gt; SEQUENCE: 56

-continued

Leu	Arg	Pro	Thr	Pro	Pro	Arg	Val	Arg	Ser	Tyr	Thr	Cys	Cys	Pro	Thr
1				5			10					15			

Pro

<210> SEQ ID NO 57  
<211> LENGTH: 18  
<212> TYPE: PRT  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Peptide linker.

&lt;400&gt; SEQUENCE: 57

Pro	Asn	Val	Ala	His	Val	Leu	Pro	Leu	Leu	Thr	Val	Pro	Trp	Asp	Asn
1					5			10				15			

Leu Arg

<210> SEQ ID NO 58  
<211> LENGTH: 18  
<212> TYPE: PRT  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Peptide linker.

&lt;400&gt; SEQUENCE: 58

Cys	Asn	Pro	Leu	Leu	Pro	Leu	Cys	Ala	Arg	Ser	Pro	Ala	Val	Arg	Thr
1					5			10			15				

Phe Pro

<210> SEQ ID NO 59  
<211> LENGTH: 28  
<212> TYPE: DNA  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: PCR primer.

&lt;400&gt; SEQUENCE: 59

cgcggtatccgtgacactc

28

<210> SEQ ID NO 60  
<211> LENGTH: 31  
<212> TYPE: DNA  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: PCR primer.

&lt;400&gt; SEQUENCE: 60

gctctagatcatttcttc tctcagtctt c

31

<210> SEQ ID NO 61  
<211> LENGTH: 15  
<212> TYPE: PRT  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Peptide linker.

&lt;400&gt; SEQUENCE: 61

Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser
1				5			10			15			

<210> SEQ ID NO 62  
<211> LENGTH: 6  
<212> TYPE: PRT  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Peptide linker

&lt;400&gt; SEQUENCE: 62

```

Ser Gly Gly Gly Gly Ser
1           5

```

&lt;210&gt; SEQ ID NO 63

&lt;211&gt; LENGTH: 14

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: artificial

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Peptide linker

&lt;400&gt; SEQUENCE: 63

```

Ala Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys Ala Gly Ser
1           5           10

```

What is claimed is:

1. A method of inhibiting growth and/or proliferation of a cancer cell, said method comprising contacting said cancer cell with a chimeric construct comprising a type I interferon attached to a full-length antibody that binds to a tumor-associated antigen, wherein said antibody is attached to said interferon by a peptide linker that is resistant to proteolysis, wherein the amino acid sequence of said peptide linker is SGGGGS (SEQ ID NO:62) or AEAAAKEAAAKAGS (SEQ ID NO:63).

2. The method of claim 1, wherein said cancer cell is selected from the group consisting of a cell in a solid tumor, a metastatic cell, a breast cancer cell, and a B cell lymphoma.

3. The method of claim 1, wherein said cancer cell is produced by a cancer selected from the group consisting of a B cell lymphoma, lung cancer, a bronchus cancer, a colorectal cancer, a prostate cancer, a breast cancer, a pancreas cancer, a stomach cancer, an ovarian cancer, a urinary bladder cancer, a brain or central nervous system cancer, a peripheral nervous system cancer, an esophageal cancer, a cervical cancer, a melanoma, a uterine or endometrial cancer, a cancer of the oral cavity or pharynx, a liver cancer, a kidney cancer, a biliary tract cancer, a small bowel or appendix cancer, a salivary gland cancer, a thyroid gland cancer, an adrenal gland cancer, an osteosarcoma, a chondrosarcoma, a liposarcoma, a testes cancer, and a malignant fibrous histiocytoma.

4. The method of claim 1, wherein said contacting comprises administration via a route selected from the group consisting of systemic administration, administration directly into a tumor site, and intravenous administration.

5. The method of claim 1, wherein said cancer cell is a cancer cell in a human.

6. The method of claim 1, wherein said cancer cell is a cancer cell in a non-human mammal.

20 7. The method of claim 1, wherein said antibody is attached to said interferon by a peptide linker wherein the amino acid sequence of said linker is SGGGGS (SEQ ID NO:62).

25 8. The method of claim 1, wherein said antibody specifically binds a tumor associated antigen selected from the group consisting of CD20, HER3, HER2/neu, mucin 1 (MUC-1), G250, mesothelin, gp100, tyrosinase, and melanoma-associated antigen (MAGE).

9. The method of claim 1, wherein said antibody is an antibody that binds CD20.

30 10. The method of claim 1, wherein said antibody is an antibody that comprises the variable regions for anti-CD20 (Rituximab).

35 11. The method of claim 1, wherein said antibody is an antibody selected from the group consisting of rituximab, IF5, B1, 1H4, CD19, B4, B43, FVS191, hLL2, LL2, RFB4, M195, HuM195, AT13/5, trastuzumab, 4D5, HuCC49, HUCC39ΔCH2 B72.3, 12C10, IG5, H23, BM-2, BM-7, 12H12, MAM-6, HMFG-1.

40 12. The method of claim 7, wherein said antibody is an antibody that binds to CD20.

13. The method of claim 7, wherein said antibody is an antibody that binds to HER2.

45 14. The method according to any one of claim 12 or 13, wherein said interferon is IFN- $\alpha$ .

15. The method according to any one of claim 12 or 13, wherein said interferon is IFN- $\beta$ .

50 16. The method of claim 1, wherein said antibody is attached to said interferon by a peptide linker wherein the amino acid sequence of said linker is AEAAAKEAAAKAGS (SEQ ID NO:63).

\* \* \* \* \*